# THESIS APPROVAL

## GRADUATE SCHOOL, KASETSART UNIVERSITY

Master of Science (Botany)
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Botany
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**TITLE:** Clarification of Taxonomic Problem in Three Groups of *Glycosmis* [*Glycosmis pentaphylla* (Retz.) DC., *G. parva* Craib and *G. mauritiana* (Lam.) Tanaka] in Thailand

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THESIS

# CLARIFICATION OF TAXONOMIC PROBLEM IN THREE GROUPS OF *GLYCOSMIS* [*Glycosmis pentaphylla* (Retz.) DC., *G. parva* Craib and *G. mauritiana* (Lam.) Tanaka] IN THAILAND

WICHAI AIYAKOOL

A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
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Wichai Aiyakool 2014: Clarification of Taxonomic Problem in Three Groups of *Glycosmis* [*Glycosmis pentaphylla* (Retz.) DC., *G. parva* Craib and *G. mauritiana* (Lam.) Tanaka] in Thailand. Master of Science (Botany), Major Field: Botany, Department of Botany. Thesis Advisor: Associate Professor Srunya Vajrodaya, Dr.rer.nat. 162 pages.

*Glycosmis pentaphylla* (Retz.) DC., *G. parva* Craib and *G. mauritiana* (Lam.) Tanaka represent the most widespread and variable species within the genus and there are still many taxonomic problems at the species level remain to be solved. Clarification of taxonomic problem in three groups of *Glycosmis* have been investigated during January 2011 – December 2013 by using morphological and chemical characters. The chemical characters of different plant parts (stem bark and leaves) of these three *Glycosmis* groups collected from different populations were compared by using chromatographic technique i.e. thin layer chromatography (TLC) and high performance liquid chromatography (HPLC).

The result of morphological study showed that within 19 populations, *Glycosmis* can be classified by ovary and leaf characters. It was shown that chemical characters in the same plant parts (stem bark and leaves) from each population of *G. parva* are similar as well as *G. mauritiana*, but there are some variation in *G. pentaphylla* collected from different populations. Thus, the chemical characters can be used in order to support the morphological characters of these three groups of *Glycosmis*, especially *G. mauritiana*.

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Wichai Aiyakool

June 2014

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# CLARIFICATION OF TAXONOMIC PROBLEM IN THREE GROUPS OF *GLYCOSMIS* [*Glycosmis pentaphylla* (Retz.) DC., *G. parva* Craib and *G. mauritiana* (Lam.) Tanaka] in Thailand

## INTRODUCTION

Thailand is rich in biological diversity consisting of species diversity, genetic diversity and habitat diversity because Thailand is situated in a tropical region. There are various types of vegetation ranging from the moist tropical evergreen forest in the south to the deciduous and upper montane forest towards the north. Thailand occupies the geographical center of the plants from Indo-Burmese elements, Indo-Chinese elements and Malesian elements, where the species richness is high of the estimated 10,000 species of vascular plants.

*Glycosmis* is a genus within the tribe Clauseneae, subfamily Citroideae in Rutaceae. Its range of distribution is centered in south and southeast Asia and extends to south China and Taiwan as well as to New Guinea and north Australia (Ridley, 1922; Swingle, 1967; Kubitzki *et al*., 2011). In Thailand, there are few references on this genus and a taxonomic revision of the genus has not been done. From the previous studies, Craib (1934) listed botanical and vernacular names of 14 species of *Glycosmis* in Thailand. The Forest Herbarium, Royal Forest Department (2001) recorded 14 species of Thai *Glycosmis* in "Thai Plant Names, Tem Smitinand, Revised Edition". Thai *Glycosmis* species are still poorly known. The actual number of existing species of *Glycosmis* in Thailand was not previously enumerated.

*Glycosmis pentaphylla* (Retz.) DC., *G. parva* Craib and *G. mauritiana* (Lam.) Tanaka represent the most widespread and variable species within this genus. Morphological characteristic varies in pair and form of foliage, size and position of inflorescence (Swingle and Reece, 1967; Stone, 1985). And there are still many taxonomic problems at the species level remain to be solved.

This present study is to compare morphological character and chemical patterns of three groups of *Glycosmis* lipophilic extracts from different organs (stem bark and leaves) in each individual and from different populations. The results of morphological character and chemical character may be useful to support the classification in species level and help in understanding ecological and chemical diversity of this species and provide a preliminary study of this genus in Thailand.

# OBJECTIVES

1. To study the morphological characters of *Glycosmis pentaphylla*, *G. parva* and *G. mauritiana* in Thailand including its geographical distribution and ecology.

2. To investigate chemical patterns in three groups of *Glycosmis* lipophilic extracts for clarifying the problems of the species identification.

# LITERATURE REVIEW

## 1. History and Classification of *Glycosmis*

The genus *Glycosmis* was first established by Corrêa in Ann. Mus. Natl. Hist. Nat. 6: 384. 1805. The type specimen is *G. arborea*. At present, *G. arborea* is treated as a synonym of *G. pentaphylla*.

*Glycosmis* were classified in subfamily Aurantioideae, tribe Clauseneae, subtribe Clauseninae in the family Rutaceae (Engler, 1931).

Swingle and Reece (1967) had classified *Glycosmis* into family Rutaceae, subfamily Aurantioideae, tribe Clauseneae. *Glycosmis* can be easily distinguished from the other genera of this tribe i.e. *Clausena, Micromelum* and *Murraya* by inflorescences which are usally covered with minute rusty-red hair.

In the field *Glycosmis* is mistaken for the genus *Aglaia* of the Meliaceae family. However, *Aglaia* can be quickly distinguished by its stellate hair or peltate scale (Stone, 1985).

A modernized treatment of subfamily Aurantioideae was given from phylogenetic base on taxonomic structure and chemical evidence. *Glycosmis* was placed in the *Bergera* Alliance, the genera in this alliance are all unarmed and bear pinnate leaves with alternate leaflet, unwinged petiole and rachis, carbazole alkaloids present but coumarins usually not present (Samuel *et al*., 2001; Mou and Zhang, 2009).

## 2. Morphologal Characters of *Glycosmis*

### 2.1 Habit

Shrubs or trees, unarmed, with rust-colored villosulous indumentum sometimes becoming bleached on terminal and axillary buds and usually on young inflorescences (Stone, 1972, 1985, 1994; Kubitzki *et al*., 2011).

### 2.2 Leaves

Leaves alternate rarely opposite, usually pinnate, 1-foliolate, or simple; leaflet 3, 5 or rarely up to 13-15 sometime varying on one plant; leaflet alternate, on short stalks, mostly somewhat leathery (Stone, 1972, 1985, 1994; Kubitzki *et al*., 2011).

### 2.3 Inflorescences and Flower

Inflorescences terminal and/or axillary, paniculate, compoundly racemose, or reduced to 1 or a few flowers.

Flowers bisexual, globose to ellipsoid in bud. Sepals 4 or 5, basally connate, petals 4 or 5, imbricate in bud, stamens 8 or 10, distinct, alternately ± unequal in length; filaments ± straight, disk annular, pulvinate, columnar, conic, or bell-shaped, gynoecium 2-5-loculed, syncarpous; radial walls of locules straight; ovules 1(or 2) per locule; style to nearly as long as ovary, persistent in fruit (Stone, 1972, 1985, 1994; Kubitzki *et al*., 2011).

2.4 Fruit and Seed

Fruit a berry, with mucilaginous pulp or dry, without pulp vesicles; endocarp membranous. Seeds with membranous seed coat; endosperm lacking; embryo straight; cotyledons elliptic, plano-convex, neither convolute nor folded; hypocotyl partly included between cotyledons (Stone, 1972, 1985, 1994; Kubitzki *et al*., 2011).

2.5 Distribution and Ecology

About 50 species, its range of distribution is hindumalayan region, centered in south and Southeast Asia and extend to South China and Taiwan as well as to New Guinea and north Australia. *G. parvifora* is well-known in cultivation and has become naturalized in tropical America and Africa (Vajrodaya, 1998; Kubitzki *et al*., 2011).

Craib (1934) listed botanical and vernacular names of 14 species of *Glycosmis* in Thailand; *G. citrifolia, G. colchinchinensis, G. crassifolia, G. dinhensis, G. malayana, G. ovoidia, G. parva, G. pentaphylla, G. puberula, G. sapindoides, G. singuliflora, G. subsessilis, G. tomentella* and *G. winitii.*

The Forest Herbarium, Royal Forest Department (2001) recorded 14 species of Thai Glycosmis in "Thai Plant Names, Tem Smitinand, Revised Edition"; *G. chlorosperma, G. colchinchinensis, G. dinhensis, G esquirolii, G. longipes, G. marcrophylla, G ovoidea, G. parkinsonii, G. parva, G. pentaphylla, G. pierrei, G. puberula, G. tricantera* and *G. trifolia*.

*Glycosmis* species occur in a wide variety of habitats. Most species are found in primary and secondary forest, evergreen or sometimes dry evergreen forest. Some species occur on limestone hill altitude 50-400 m.

## 2.6 Morphology of *Glycosmis pentaphylla*

Shrub or small tree to 5 m tall, unarmed, evergreen, aromatic, young shoot covered with dense fine-woolly rust-colored (Swingle and Reece, 1967; Stone 1972; Dianxiang and Hartley, 2008).

Leaves alternate odd-pinnate or unifoliolate, petiole 2-10 cm, leaflet blade ovate to elliptic 8-25 by 2-7 cm, papery base cuneate, margin serrate, apex mucronate (Stone 1972; Dianxiang and Hartley, 2008). Leaves are very variable in pair and form of foliage (Swingle and Reece, 1967).

Inflorescence terminal and/or axillary, paniculate. Flower bisexual, globose to ellipsoid in bud, sepals 5, basally connate, broadly ovate, less than 1 mm; petals 5, imbricate in bud, 3-4 mm, caducous; stamens 10, distinct, alternately unequal in length; filaments straight; disk annular shaped; ovary superior, globose to broadly ovoid, glabrous; style to nearly as long as ovary, persistent in fruit. Fruit a berry, reddish, subglobose, 8-10 mm in diamiter. Seed 2 perfruit (Swingle and Reece, 1967; Stone 1972; Dianxiang and Hartley, 2008).

Type specimens.— India, Koenig (LD)

Distribution.— Widespread in India (type), Ceylon, Burma, Thailand, S China, Indochina (Stone, 1985), Malay peninsula to North Australia, Philippines and Java (Craib, 1934; Swingle and Reece, 1967; Stone 1972).

2.7 Morphology *Glycosmis parva*

Shrub 50-100 cm high, unarmed, young shoot covered with dense fine-woolly rust-colored, young stem sometime angular (Craib, 1926; Guillaumin, 1946).

Leaves unifoliolate; alternate sometime subopposite; petiole 2-6 mm; leaf blade narrowly elliptic, glabrous on both surface, papery, dark green, base cuneate, margin entire, apex obtuse, 3.5-9 by 1-2.5 cm; lateral veins 11-14 pairs (Craib, 1926; Guillaumin, 1946).

Flower solitary, axillary and terminal, sepal 5, broadly ovate, glabrous both surface, less than 1 mm; petal 5,ovate, white, carducous, imbricate in bud, 2-4 mm; stamen 10, filament ca. 1.0 mm linear; ovary superior, ovoid, glabrous, 1.5 mm long; style to nearly as long as ovary, persistent in fruit. Fruit berry, green, pink when mature, globose to ovate, 0.7-1.0 cm long; one seed per fruit (Craib, 1926; Guillaumin, 1946).

Type specimens.— Thailand, Sakeo, Krabin, Kerr 9766 (K) (Appendix Figure 1)

Distribution.— Thailand (type) (Craib, 1926, 1934; Stone, 1985)

2.7 Morphology *Glycosmis mauritiana*

Shrub to small tree 50-150cm high, evergreen, aromatic, young shoot covered with dense fine-woolly rust-colored (Swingle and Reece, 1967).

Leaves 3-5 foliolate, alternate; petiole 2.0-5.0 cm; leaflet blade ovate to elliptic, glabrous on both surface, papery, dark green, base obtuse, margin entire, apex acute, 9-16.5 x 3.5-5 cm, lateral veins 5-6 pairs (Swingle and Reece, 1967; Stone, 1994).

Inflorescence or solitary flower, axillary, covered with dense fine-woolly rust-colored when young. Flower globose in bud, 5 merous, covered with dense fine-woolly rust-colored, sepal 5, broadly ovate, puberulous outside, glabrous inside, less than 1 mm; petal 5, white, ovate, glabrous always puberulent both surface, caducous, 2.0-3.0 mm; stamen 10, filament ca. 1.0 mm, linear, puberulent, anther elliptic; ovary superior, ovoid, puberulent, style extreamly short. Fruit berry, pink, green, 0.8-1.5 cm in diam; seed 2 per fruit (Swingle and Reece, 1967, Stone, 1994).

Type specimens.— Malaysia, Kedah, Ridley 15467 (K) (Appendix Figure 2)

Distribution.— South India to Ceylon, Andaman and Nicobar Island, Burma, Thailand, Malaysia (type) (Swingle and Reece, 1967; Stone, 1985, 1994).

**4. Phytochemistry**

Based on phytochemical comparisons between many *Glycosmis* species collected in Thailand, compounds of 6 classes were show to play a prominent role in the genus: the acridone, carbazole, quinolone and quinazoline alkaloids together with flavanone and amides. Amides, particularly a number of sulphur containing derivative represent typical character of the leaves, whereas the bark mainly accumulates carbazoles and acridones, and the roots acridones and quinolones (Vajrodaya, 1998; Vajrodaya *et al*., 1998).

4.1 *Glycosmis pentaphylla*

Some of the major classes of compounds reported from *G. pentaphylla* include alkaloids (Table 1), flavonoids (Table 2) and amides (Table 3).

Phytochemicals such as arborinine, glycozolicine, 3- formyl carbazole, glycosinine, mupamine, varbazole, 3- methyl carbazole, glycolone, glycozolidol, glycozolinine, glycophymoline, glycophymine, glycomide, glycozoline, noracronycine, have been reported from this plant (Table 1) (Greger *et al*., 1992; Greger *et al*., 1993; Ono *et al*., 1995; Tian *et al*., 1995; Chakravarty *et al*., 1996; Wang *et al*., 2005; Sreejith *et al*., 2012).

Leaves of *G. pentaphylla* yielded two furoquinoline, kokusaginine and skimmianine. Other alkaloids reported from the leaves include glycosine, arborine, glycosminine, arborinine, glycosamine, glycorine and glycosmicine. Roots of *G. pentaphylla* contain the carbazole alkaloids, glycozolicine, 3-formylcarbazole, glycosinine, glycozoline, glycozolidine, skimmianine, fagarine and dictamine. Stems of G. *pentaphylla* contain arborinine; other minor alkaloids also occur in this plant (Ono *et al*., 1995; Tian *et al*., 1995; Wang *et al*., 2005).

4.2 *Glycosmis parva*

The major compounds of *G. parva* has been reported are quinolone, quinazoline, furoquinoline, carbazole, acridone types of alkaloids (Table 4) and also sulfur-containing amides (Table 5).

Chansriniyom *et al.* (2009) reported the structure elucidation of a new acridone alkaloid, form branch and leaves of *G. parva*: glycosparvarine, two new N-[(4-monoterpenyloxy) phenylethyl]-substituted sulfur-containing propanamide derivatives, S-deoxydihydroglyparvin and S-deoxytetrahydroglyparvin, which were isolated together with nine known compounds, and the anti-HSV activity of the isolates.

4.3 *Glycosmis mauritiana*

Leaves of *G. mauritiana* revealed the presence of carbohydrates, alkaloids, flavonoids, phenols and tannins in all the tested extracts. Saponins were present in petroleum ether extract, steroids in ethylacetate and methanol extracts and proteins in ethylacetate and water extracts (Ebenezer *et al.*, 2013b).

Intekhab *et al*. (2010) isolated and characterized flavone glycoside from ethyl acetate extract of the aerial parts of *G. mauritiana*.

From phytochemical investigation from *G. mauritiana* revealed the presence of alkaloids (Table 6), flavonoids (7) and amides (8).

**Table 1** Alkaloids from *G. pentaphylla*

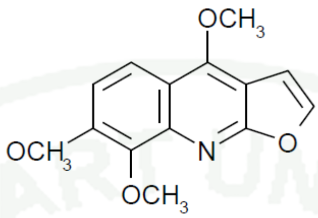
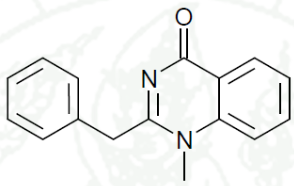
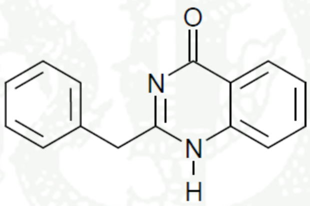
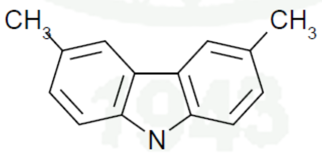
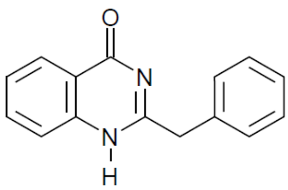
| Plant part | Compounds | References |
|---|---|---|
| Leaves |  Skimmianine | Chatterjee and Ghosh, 1954 |
| Leaves |  Glycosminine | Chatterjee and Ghosh, 1954 |
| Leaves |  Glycosine | Chatterjee and Ghosh, 1954 |
| Stem bark<br>Root |  Glycozoline | Chakraborty, 1969; Chakravarty *et al.*, 1999 |
| Flower |  Glycophymine | Sarkar and Chakraborty, 1977; 1978 |

**Table 1** (Continued)

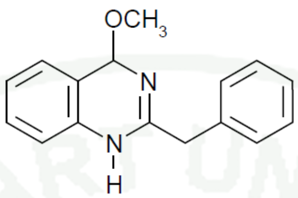
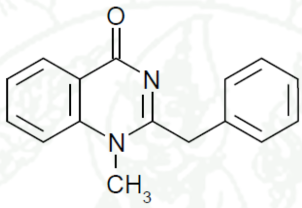
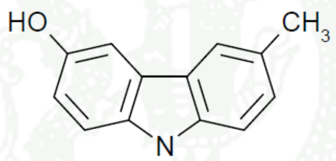
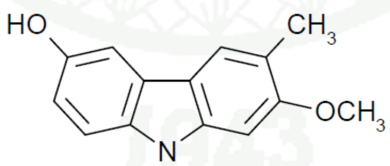
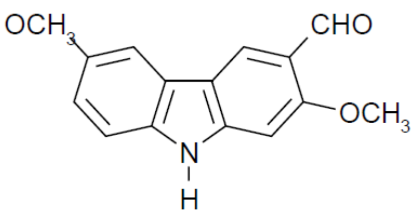
| Plant part | Compounds | References |
|---|---|---|
| Flower |  Glyphymoline | Sarkar and Chakraborty, 1979 |
| Flower<br>Leaves<br>Stem |  Arborine | Sarkar and Chakraborty, 1979<br>Ahmad *et al*., 1996;<br>Vajrodaya, 1998;<br>Ito *et al*., 2004 |
| Seed |  Glycozolinine | Mukherjee *et al*., 1982 |
| Root |  Glycozolidal | Bhattacharyya *et al*., 1985 |
| Root |  Glycozolidine | Bhattacharyya, 1985;<br>Chakravarty *et al*., 1999 |

**Table 1** (Continued)

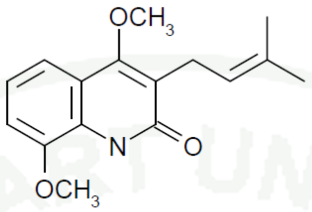
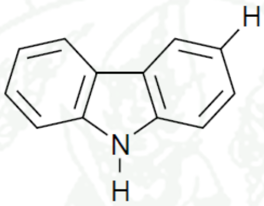
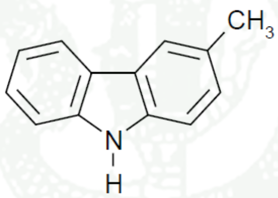
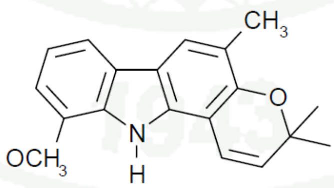
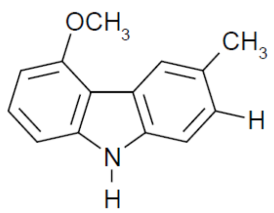
| Plant part | Compounds | References |
|---|---|---|
| Leaves | <br>Glycolone | Bhattacharyya and Chowdhury, 1985 |
| Root | <br>Carbazole | Chowdhury *et al*., 1987 |
| Root | <br>3-Methylcarbazole | Chowdhury *et al*., 1987 |
| Leaves | <br>Mupamine | Roy and Chakraborty, 1989 |
| Root | <br>Glycozolicine | Jash *et al*., 1992 |

**Table 1** (Continued)

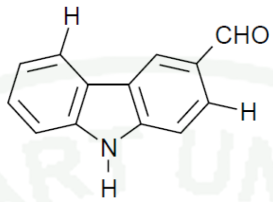
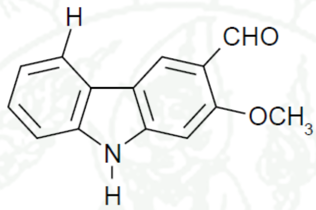
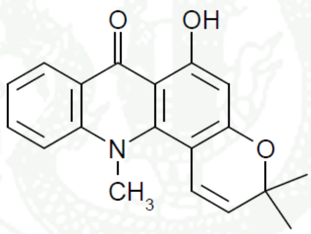
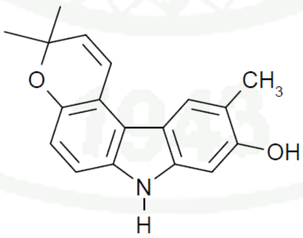
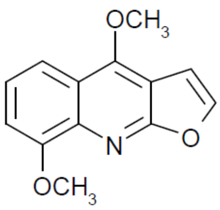
| Plant part | Compounds | References |
|---|---|---|
| Root |  3-Formyl carbazole | Jash *et al*., 1992 |
| Root |  Glycosinine | Jash *et al*., 1992 |
| Root Bark |  Noracronycine | Vajrodaya, 1998 |
| Stem Bark |  2-Hydroxyglycocomaurin | Vajrodaya, 1998 |
| Root Bark |  γ-Fagarine | Vajrodaya, 1998 |

**Table 1** (Continued)

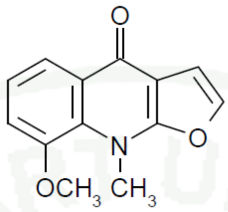
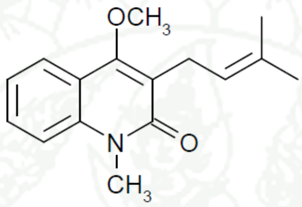
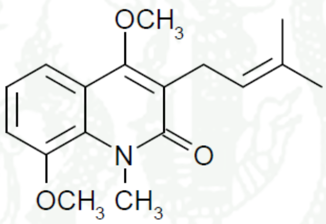
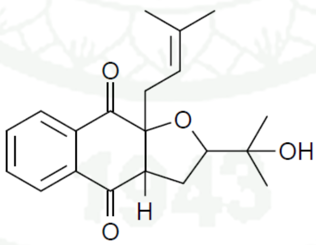
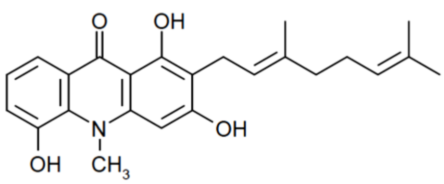
| Plant part | Compounds | References |
|---|---|---|
| Root Bark |  Iso-γ-Fagarine | Vajrodaya, 1998 |
| Root Bark |  N-Methylatanine | Vajrodaya, 1998 |
| Root Bark |  O-Methylglycosolone | Vajrodaya, 1998 |
| Stem bark |  Glycoquinone | Ito *et al*., 1999 |
| Stem bark |  Glycocitrine-III | Ito *et al*., 1999 |

**Table 1** (Continued)

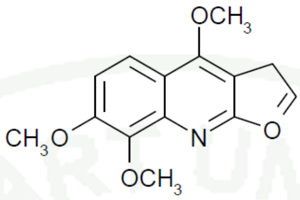
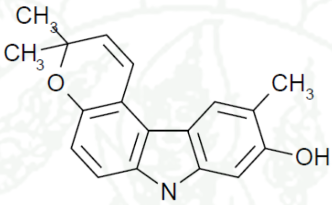
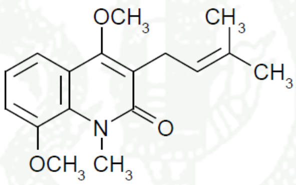
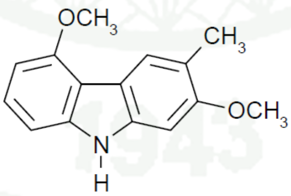
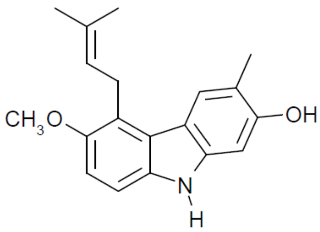
| Plant part | Compounds | References |
|---|---|---|
| Stem<br>Root | <br>Shikimmianine | Chakravarty *et al.*, 1999; Yang *et al.*, 2012 |
| Root | <br>Glycoborinine | Chakravarty *et al.*, 1999 |
| Root | <br>3-(3’,3’-dimethyl-allyl) -4,8-dimethoxy-*N*-methyl quinolin-2-one | Chakravarty *et al.*, 1999 |
| Stem | <br>Glybomine A | Ito *et al.*, 2004 |
| Stem | <br>Glybomine B | Ito *et al.*, 2004; Yang *et al.*, 2012 |

**Table 1** (Continued)

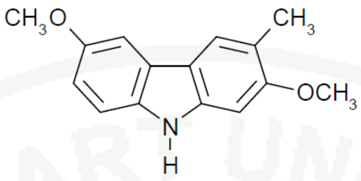
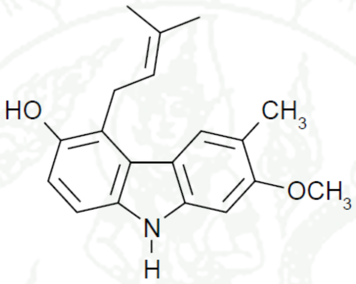
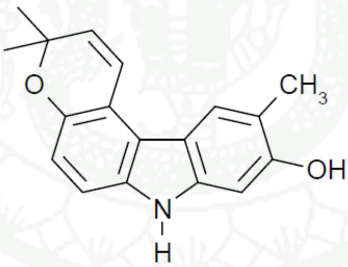
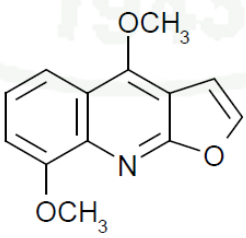
| Plant part | Compounds | References |
|---|---|---|
| Stem |  Gycozolidine | Ito *et al.*, 2004 |
| Stem |  Glybomine C | Ito *et al.*, 2004 |
| Stem |  Glycoborinine | Ito *et al.*, 2004 |
| Stem |  4,8-dimethoxyfuro[2,3-*b*]quinoline | Ito *et al.*, 2004 |

**Table 1** (Continued)

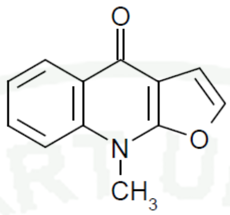
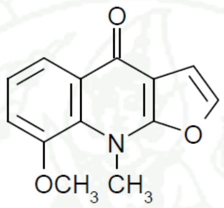
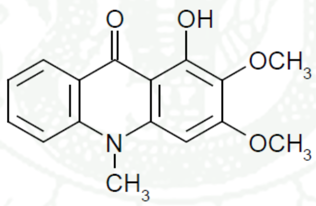
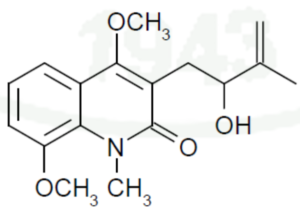
| Plant part | Compounds | References |
|---|---|---|
| Stem |  Isodictamnine | Ito *et al*., 2004 |
| Stem Bark |  Iso-γ-fagarine | Vajrodaya, 1998; Ito *et al*., 2004 |
| Stem Bark |  Arborinine | Ito *et al*., 2004; Vajrodaya, 1998; Yang *et al*., 2012 |
| Fruit |  Glycopentaphyllone | Sripisut *et al*., 2012 |

**Table 1** (Continued)

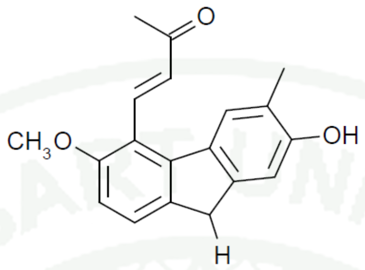
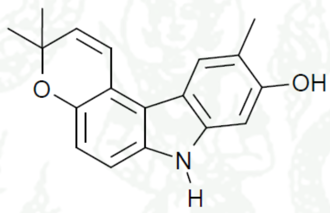
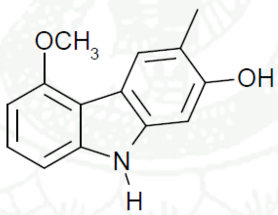
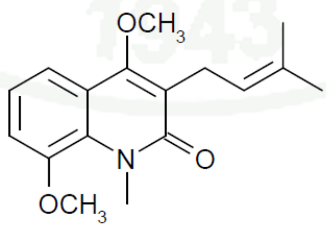
| Plant part | Compounds | References |
|---|---|---|
| Stem | <br>4-(7-hydroxy-3-methoxy-6-methyl-9H-carbazol-4-yl)but-3-en-2-one | Yang *et al*., 2012 |
| Stem | <br>Glycoborinine | Yang *et al*., 2012 |
| Stem | <br>Carbalexine A | Yang *et al*., 2012 |
| Stem | <br>4,8-dimethoxy-1-methyl-3-(3-methylbut-2-en-1-yl)quinolin-2(1*H*)-one | Yang *et al*., 2012 |

**Table 1** (Continued)

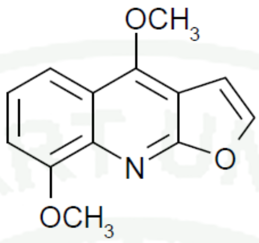
| Plant part | Compounds | References |
|---|---|---|
| Stem | <br>4,8-dimethoxyfuro[2,3-*b*]quinoline | Yang *et al*., 2012 |

**Table 2** Flavonoids from *G. pentaphylla*

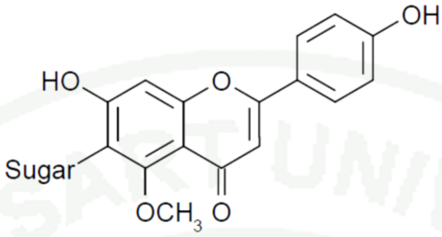
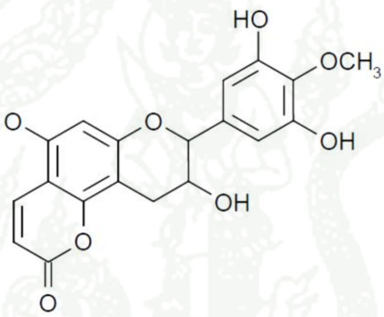
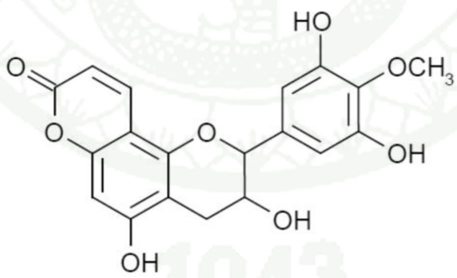
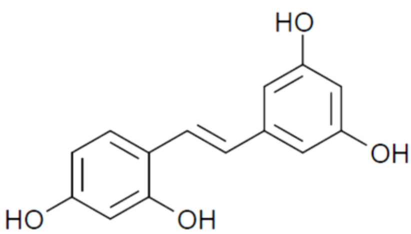
| Plant part | Compounds | References |
|---|---|---|
| Leaves |  7,40-dihydroxy-5-methoxyflavone-6-C-b-D-glucopyranoside | Sharma *et al*., 2010 |
| Stem |  glycoflavanones A | Wu *et al.*, 2012 |
| Stem |  glycoflavanones B | Wu *et al.*, 2012 |
| Stem |  oxyresveratrol | Wu *et al.*, 2012 |

**Table 2** (Continued)

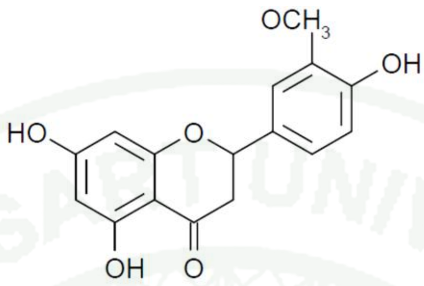
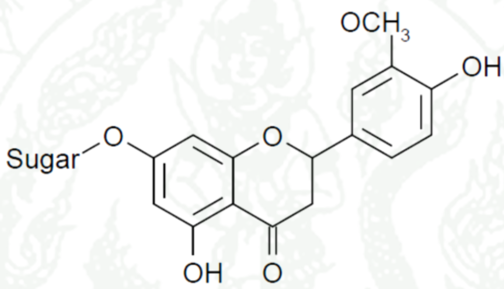
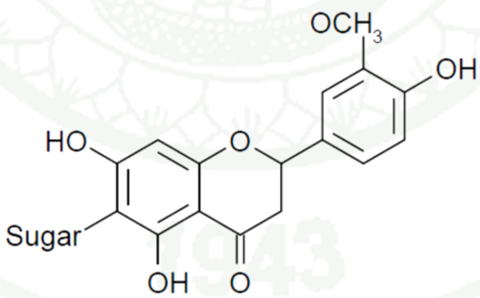
| Plant part | Compounds | References |
|---|---|---|
| Leaves | <br>5,7,40-trihydroxy-30-methoxy flavone | Khan *et al*., 2013 |
| Leaves | <br>5,40-dihydroxy-30-methoxy-7-O-β-D-glucupyranosyl flavanone | Khan *et al*., 2013 |
| Leaves | <br>5,7-dihydroxy-2-[4-hydroxy-3-(methoxymethyl)phenyl]-6-C-β-D-glucopyranosyl flavone | Khan *et al*., 2013 |

**Table 3** Amides from *G. pentaphylla*

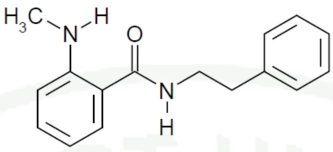
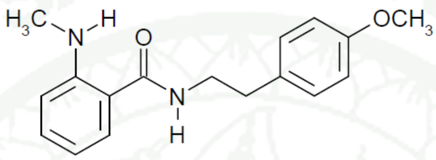
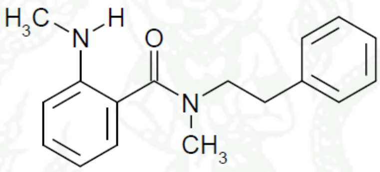
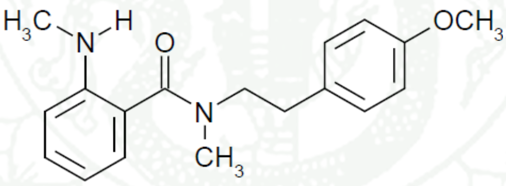
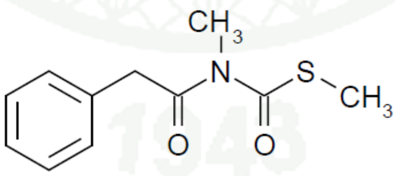
| Plant part | Compounds | References |
|---|---|---|
| Stem Bark<br>Leaves | <br>N-Methyldoisuthine | Vajrodaya, 1998 |
| Leaves | <br>N-Demethylmethoxydoisuthine | Vajrodaya, 1998 |
| Stem Bark<br>Root Bark<br>Leaves | <br>Doisuthine | Vajrodaya, 1998 |
| Stem Bark<br>Leaves | <br>Methoxydoisuthine | Vajrodaya, 1998 |
| Stem Bark | <br>Ritigalin | Vajrodaya, 1998 |

**Table 4** Alkaloids from *G. parva*

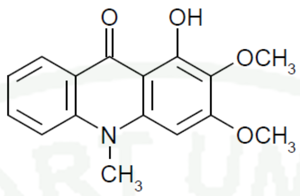
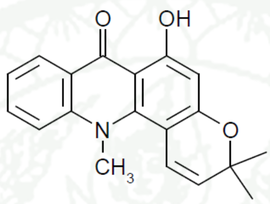
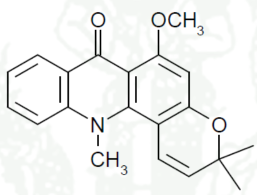
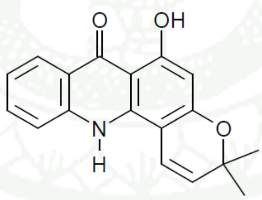
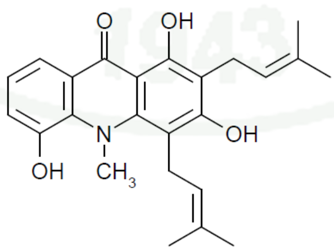
| Plant part | Compounds | References |
|---|---|---|
| Leaves<br>Root Bark | <br>Arborinine | Vajrodaya, 1998 |
| Root Bark | <br>Noracronycine | Vajrodaya, 1998 |
| Root Bark | <br>N-Demethylaronycine | Vajrodaya, 1998 |
| Stem bark<br>Root Bark | <br>N-Demethylnoracronycine | Vajrodaya, 1998 |
| Stem bark | <br>N-Methylatalaphylline | Vajrodaya, 1998 |

**Table 4** (Continued)

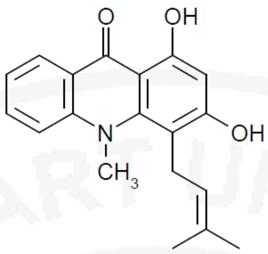
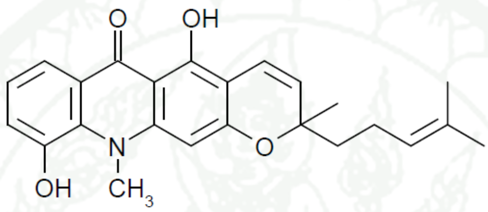
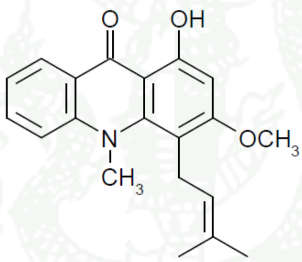
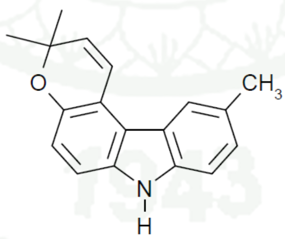
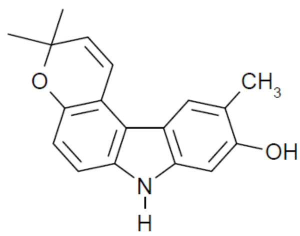
| Plant part | Compounds | References |
|---|---|---|
| Root Bark | <br>Glycocitrine II | Vajrodaya, 1998 |
| Stem Bark | <br>Glycofoline | Vajrodaya, 1998 |
| Root Bark | <br>3-O-Methylglycocitrine II | Vajrodaya, 1998 |
| Stem bark<br>Root Bark | <br>Glycomaurin | Vajrodaya, 1998 |
| Stem bark | <br>2-Hydroxyglycomaurin | Vajrodaya, 1998 |

**Table 4** (Continued)

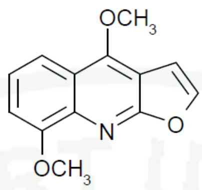
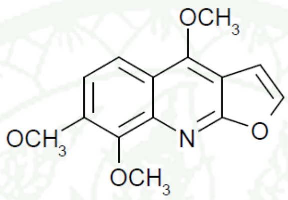
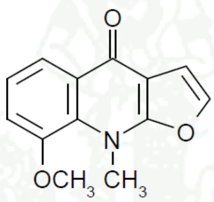
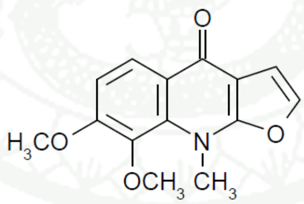
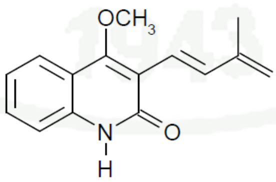
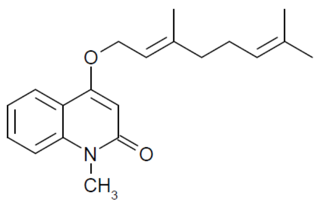
| Plant part | Compounds | References |
|---|---|---|
| Root Bark | <br>γ-fagarine | Vajrodaya, 1998 |
| Root Bark | <br>Skimmianine | Vajrodaya, 1998 |
| Root Bark | <br>Iso-γ-fagarine | Vajrodaya, 1998 |
| Leaves<br>Root Bark | <br>Isoskimmianine | Vajrodaya, 1998 |
| Leaves | <br>Schinifolin | Vajrodaya, 1998 |
| Root Bark | <br>4-Geranyloxy-N-methyl-quinolone | Vajrodaya, 1998 |

**Table 4** (Continued)

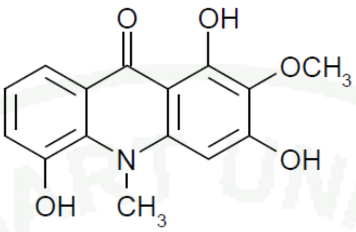
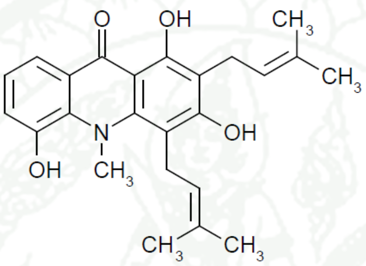
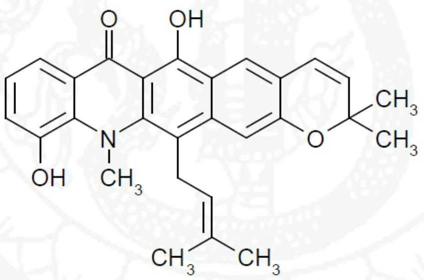
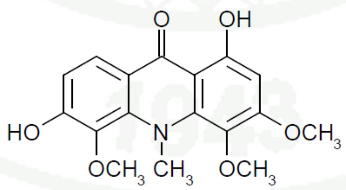
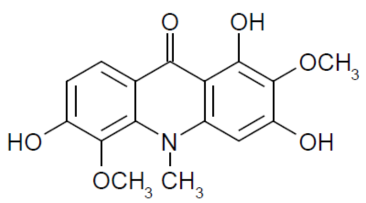
| Plant part | Compounds | References |
|---|---|---|
| Leaves |  Glycoparvarine | Chansriniyom *et al.*, 2009 |
| Leaves |  Acridone | Chansriniyom *et al.*, 2009 |
| Leaves |  *N*-methylcyclo-atalaphylline-A | Chansriniyom *et al.*, 2009 |
| Leaves |  Glycofolinine | Chansriniyom *et al.*, 2009 |
| Leaves |  Citramine | Chansriniyom *et al.*, 2009 |

**Table 5** Amides from *G. parva*

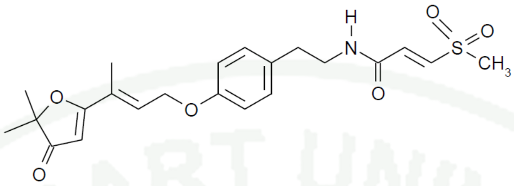
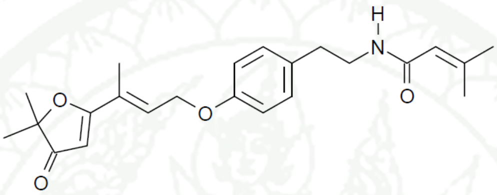
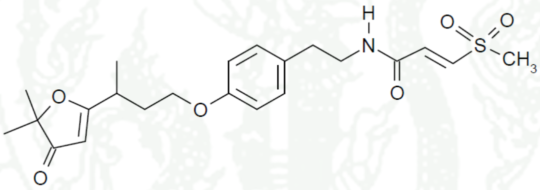
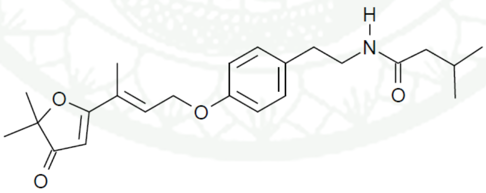
| Plant part | Compounds | References |
|---|---|---|
| Leaves | <br>Glyparvin-A | Vajrodaya, 1998 |
| Leaves | <br>Puchinamide | Vajrodaya, 1998 |
| Leaves | <br>Dihydroglygoparvin | Vajrodaya, 1998 |
| Leaves | <br>Khaochamide | Vajrodaya, 1998 |

**Table 6** Alkaloids from *G. mauritiana*

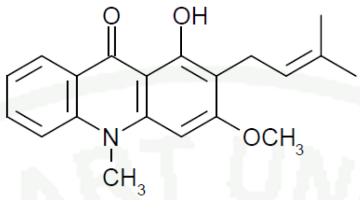
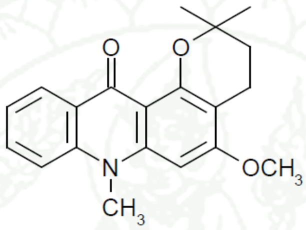
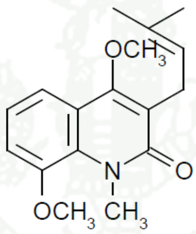
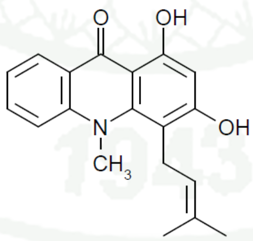
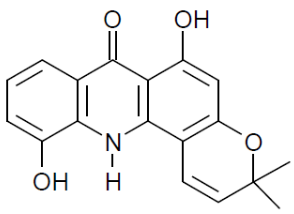
| Plant part | Compounds | References |
|---|---|---|
| Root | <br>1-hydroxy-3-methoxy-2-(3-methylbut-2-enyl)-N-methylacridan-9-one | Rastogi *et al.*, 1979 |
| Root | <br>Pyrano-[2, 3-a]-acridine | Rastogi *et al.*, 1979 |
| Root | <br>4,8-dimethoxy-3-(3-methylbut-2-enyl)-N-methyl-2-quinolone | Rastogi *et al.*, 1979 |
| Root Bark | <br>Glycocitrine II | Vajrodaya, 1998 |
| Stem Bark | <br>Ataphyllidine | Vajrodaya, 1998 |

**Table 6** (Continued)

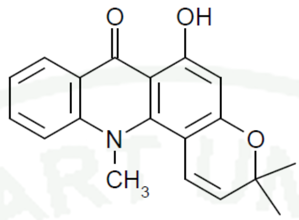
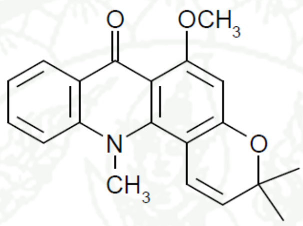
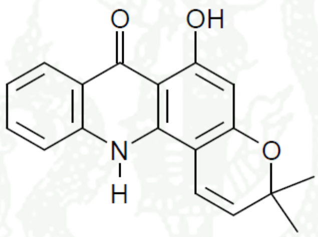
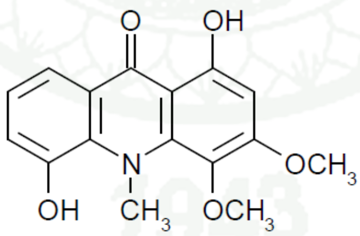
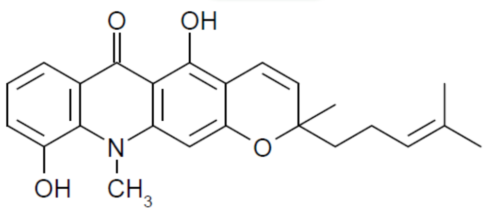
| Plant part | Compounds | References |
|---|---|---|
| Root Bark |  Noracronycine | Vajrodaya, 1998 |
| Stem Bark<br>Root Bark |  N-Demethylacronycine | Vajrodaya, 1998 |
| Root Bark |  N-Demethylnoracronycine | Vajrodaya, 1998 |
| Stem Bark |  Citrusnine I | Vajrodaya, 1998 |
| Stem Bark |  Glycofoline | Vajrodaya, 1998 |

**Table 6** (Continued)

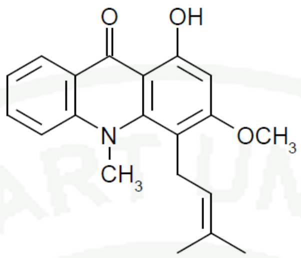
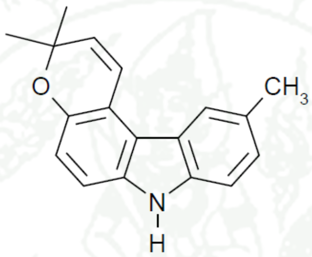
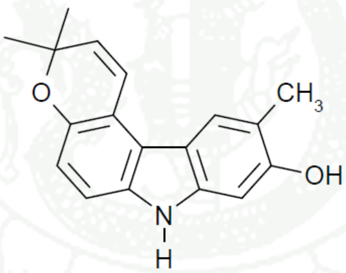
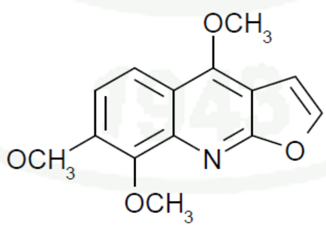
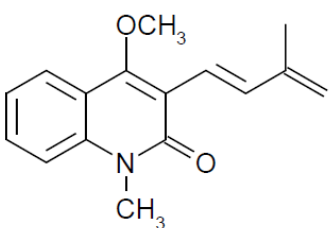
| Plant part | Compounds | References |
|---|---|---|
| Root Bark |  3-O-Methylglycocitrine II | Vajrodaya, 1998 |
| Stem Bark<br>Root Bark |  Glycomaurin | Vajrodaya, 1998 |
| Stem Bark |  2-Hydroxyglycomourin | Vajrodaya, 1998 |
| Root Bark |  Skimmianine | Vajrodaya, 1998 |
| Root Bark |  N-Methylschinifolin | Vajrodaya, 1998 |

**Table 6** (Continued)

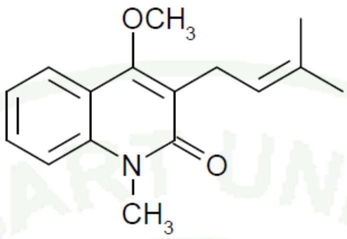
| Plant part | Compounds | References |
|---|---|---|
| Root Bark |  N-Methylatalanine | Vajrodaya, 1998 |

**Table 7** Flavonoids from *G. mauritiana*

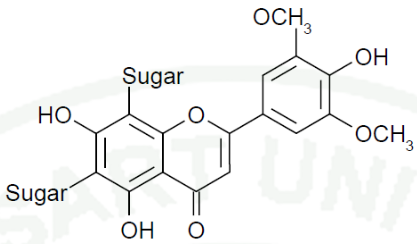
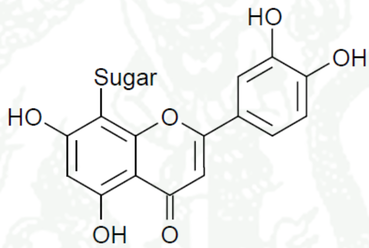
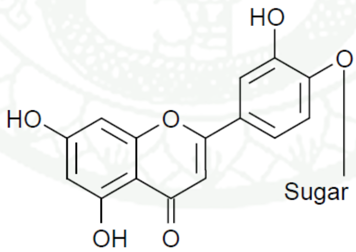
| Plant part | Compounds | References |
|---|---|---|
| Aerial Part |   5,7,4'-trihydroxy-3',5'-dimethoxyflavone-6-C-α-L-arabinopyranoside-8-C- β -D-glucopyranosyl(1 → 200)-glucopyranoside | Intekhab and Aslam, 2010a |
| Root |   luteolin 8-C- β -D-glucopyranoside | Intekhab and Aslam, 2010b |
| Aerial Part |   Luteolin-4'-O-[α-L-rhamnopyranosyl-(1→2)-{α-L-rhamnopyra- nosyl-(1→6)}-β-D-glycopyranoside] | Intekhab *et al*., 2011 |

**Table 8** Amides from *G. mauritiana*

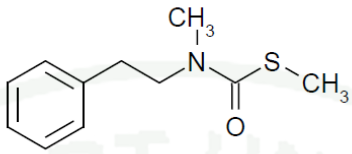
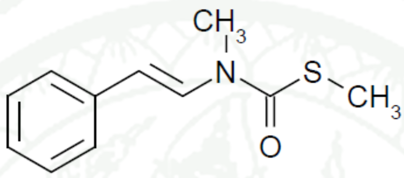
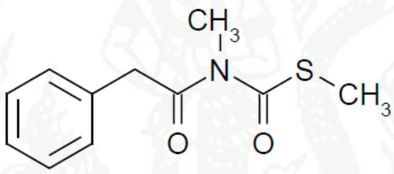
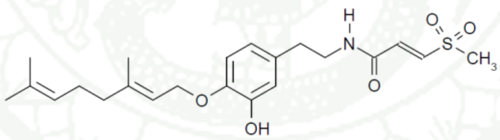
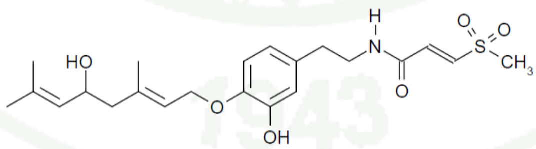
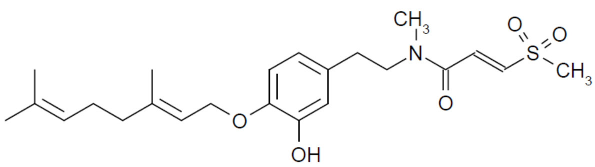
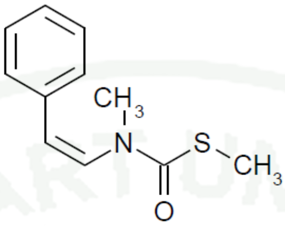
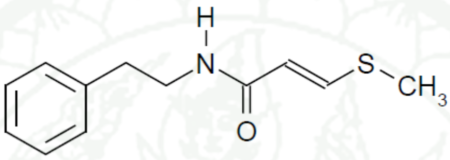
| Plant part | Compounds | References |
|---|---|---|
| Leaves |  Niranin | Greger *et al.*, 1996; Vajrodaya, 1998 |
| Leaves |  Dehydroniranin-A | Greger *et al.*, 1996; Vajrodaya, 1998 |
| Leaves |  Ritigalin | Greger *et al.*, 1996; Vajrodaya, 1998 |
| Leaves |  Sakerine | Vajrodaya, 1998 |
| Leaves |  Sakerol | Vajrodaya, 1998 |
| Leaves |  Methylgerambullin | Vajrodaya, 1998 |

**Table 8** (Continued)

| Plant part | Compounds | References |
|---|---|---|
| Leaves |  Dehydroniranin | Vajrodaya, 1998 |
| Leaves |  Sinharin | Vajrodaya, 1998 |

**6. Uses**

*Glycosmis* species was beneficial in conventional medicine all over the tropical areas. Plants of this genus are used as a traditional medicine for treatment of various diseases. The genus is a rich source of quinolone, quinazoline, furoquinoline, carbazole, acridone types of alkaloids, and also sulfur-containing amides, and flavonoids (Vajrodaya, 1998; Sreejith *et al.*, 2012; Sarkar *et al.*, 2013).

*G. pentaphylla* is taken to treat bilious attacks. Decoction of roots and leaves is taken for intestinal trouble. The plant is used for cough, rheumatism, anaemia and jaundice. Stems and roots of plant are used for treatment of ulcer. Paste of leaves, with a bit of ginger, applied over the navel for worms and other bowel disorders. It has been used as folk medicine for the treatment of fever, liver, complaints and certain other diseases (Wang *et al*., 2005; Sreejith *et al*., 2012).

*G. mauritiana*, its traditional usages for various ailments such as roots for dysentery and snake bite leaves for gastritis, headache, eczema and skin diseases by different tribals of India (Ebenezer *et al*., 2013a). In Tamil Nadu, crushed leaves and bark of *G. mauritiana* were externally applied on forehead to cure severe headache by the Irula tribes of the Nilgiris; leaf paste was externally used for eczema and skin diseases by the Thottianaickan tribes of Semmalai hills and Valaiyans tribes of Piranmalai hills (Intekhab *et al.*, 2011; Ebenezer *et al.*, 2013b).

# MATERAILS AND METHODS

## Materials

### 1. Plant Materials

Three species of *Glycosmis* were collected from different provinces of Thailand. The locations of collecting are outlined below (Figure 1).

1.1 Glycosmis pentaphylla

EASTERN: Nakhon Ratchasima

CENTRAL: Saraburi, Nakhon Nayok and Bangkok

SOUTHEASTERN: Chon Buri, Rayong, Chanthaburi, and Trat

PENINSULAR: Phangnga, Trang, Krabi, Nakhon Si Thammarat, Satun and Songkhla

1.2 Glycosmis parva

EASTERN: Nakhon Ratchasima (Sakaerat, Haew Narok Waterfall)

SOUTHEASTERN: Rayong

1.3 Glycosmis mauritiana

SOUTHWESTERN: Prachuap Khiri Khan (Trail to Praya Nakhon Cave and Laem Sala)

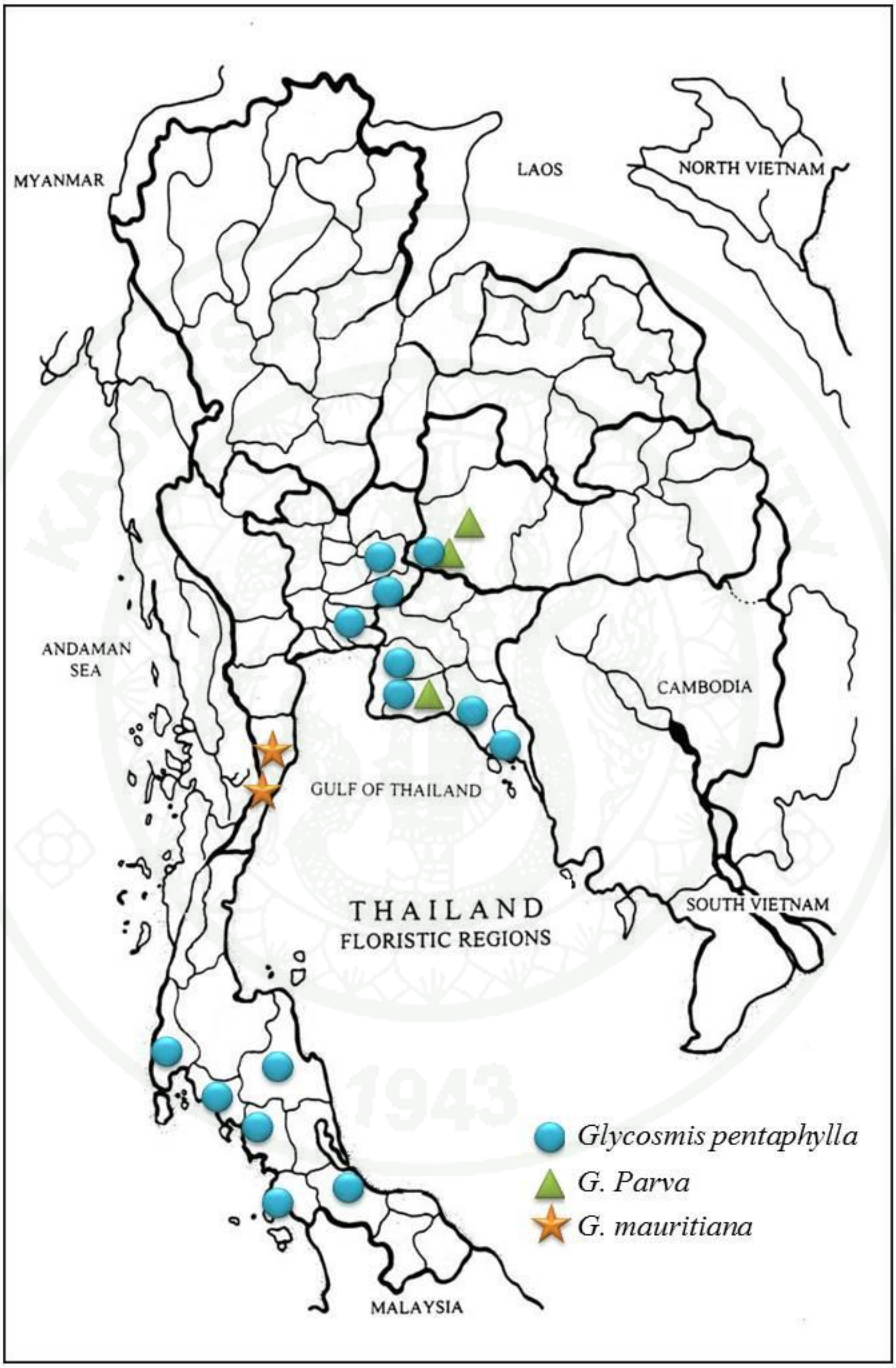



**Figure 1** Field collection sites in Thailand

**2. Field collecting and taxonomic study material**

Plant press, pruning knife, plastic bags, newspapers, tags, field book, pencil, camera, alcohol 70%, mounting papers (papers of 300 gram of 42 x 26.5 cm, covers of 42 x 27 cm, brown covers of 42 x 27.5 cm), needle and thread, labels of 13.5 x 10 cm and glue, stereo microscope, petri dish, and dissecting needle.

**3. Instrumentation**

3.1 Labatory Instruments

The instruments in this study are rotary evaporator (Buchi Rotavapor R-114, R-205), UV cabinet (CN-6.T) with Ultraviolet radiation obligatory eye protection 254 nm and 365 nm (Vilber Lourmal serial number V01 5636), analyical balance (Mettler Toledo AG 204), oven (National EH 5741), deep freeze (Sanyo -85 ˚C), blender, desiccator, TCL tank, filter paper (110 mm and 185 mm Ø Whatman No. 1) and glasswares such as pipettes, erlenmeyer flask, Buchner funnel, separatory funnel, 6´´ diameter Petri dish, volumetric flask, beaker.

3.2 Chromatographic Techniques

3.2.1 Thin layer chromatography (TLC)

| | |
|---|---|
| Technique | : one way, ascending |
| Absorbent | : silica gel 60 $F_{254}$ (0.2 mm thickness, Merck) |
| Plate size | : 20 x 20 $cm^2$ |
| Solvent system | : haxane:ethylacetate (7:3) |
| Distance | : 15 cm |
| Temperature | : 25-30 ºC |

### 3.2.2 High Performance Liquid Chromatography (HPLC)

| | |
|---|---|
| HPLC technology | : Agilent 1100 series |
| Detector | : UV photodiode array detector 230 nm wave length |
| Column | : reverse phase ChromSepher 5 $C_{18}$ column (250 x 4.6 mm; part number 28105-254630) |
| Sample | : 10 mg/ml of lipophilic extract filtered with 13 mm x 0.45 µm Nylon filter |
| Injection | : 20 µl |
| Flow rate | : 1.0 ml/min |
| Time | : 30 mins |
| Solvent system | : methanol gradient 60%-100% (HPLC grade Merck) in aqueous buffer (0.015 M tetrabutyl ammonium hydroxide ($C_{16}H_{37}NO$, AR grade Fluka) and 0.015 M ortho-phosphoric acid (AR grade Merck), pH 3) |

Mobile phase :

| Time (Min) | MeOH | Buffer |
|---|---|---|
| 0.10 | 60 | 40 |
| 17.00 | 90 | 10 |
| 22.00 | 100 | 0 |
| 28.00 | 100 | 0 |
| 29.00 | 60 | 40 |

**4. Chemicals**

The organic solvents utilized in extraction were methanol ($CH_3OH$: AR grade Merck), chloroform ($CHCl_3$: AR grade Merck) and distilled water ($H_2O$: 16 MΩ /cm, Millipore). The chemicals for chromatography were ethyl acetate ($CH_3COOC_2H_5$: AR grade Merck, Fisher) and hexane ($CH_3(CH_2)_4CH_3$: AR grade Merck, Labscan).

The reagent for phytochemical screening were bismuth subnitrate [$BiO(NO_3)$ $H_2O$: (AR grade Merck)], glacial acetic acid ($CH_3COOH$: AR grade Merck), distilled water ($H_2O$: 16 MΩ/cm Millipore) and potassium iodide (KI: AR grade Merck)), 10% Sodium hydroxide (NaOH: AR grade Mallinckrodt), 97% sulfuric acid ($H_2SO_4$ : AR grade Fisher Scientific), anisaldehyde ($C_8H_8O_2$: AR grade Fluka).

## Methods

**1. Literature review and Herbarium Study**

The literature review of the *G. pentaphylla*, *G. parva* and *G. mauritiana* were collected from secondary sources including floras, journals and reports on the survey of this genus in various sites were collected and compiled. Voucher specimens were compared with herbarium specimens deposit at the Forest Herbarium (BKF), National Park Wildlife and Plants Conservation Department, Bangkok Herbarium, Department of Agriculture (BK), Queen Sirikit Botanic Garden Herbarium (QBG) Prince of Songkhla University Herbarium (PSU), the herbarium of Faculty of Science, Khon Kaen University (KKU) and Professor Kasin Suvatabhandhu Herbarium, Chulalongkorn University (CU) were also studied.

**2. Field Surveys and Specimen Collections**

Field surveys and specimens of *G. pentaphylla*, *G. parva* and *G. mauritiana* were collected from from different populations. Three duplicates of flowering or fruiting specimens were used. Field observations such as some morphological characters, ecological and phenological information localities and their uses are recorded.

The stem bark and leaves were collected and dried at room temperature, the dried samples were chopped into small pieces and grinded for phytochemical investigation.

**3. Morphological and Taxonomic Studies**

Morphological characteristics of *G. pentaphylla*, *G. parva* and *G. mauritiana* were observed from the fresh materials. Flowers were dissected and examined under stereo microscope and also photographs as well as measurement of various parts of plants were taken. The detailed descriptions of each populations as well as line drawing were provided for clarification. The dried specimens were mounted on herbarium sheets, labeled and deposited in Herbarium of the Department of Botany, Kasetsart University.

**4. Phytochemical investigation**

4.1 Extraction and Separation

The homogenized bark and leaves (5 g) were extracted with methanol at room temperature for 7 days, filtered and concentrated. The aqueous residues were extracted with chloroform ($CHCl_3$) and the chloroform fractions were evaporated to dryness and dissolved in methanol for further analyses.

## 4.2 Chromatographic analyses

### 4.2.1 TLC screening

The TLC plates were observed under UV light (254 and 365 nm) and sprayed with specific reagents for detection of major groups of compounds. Color spots were observed and calculated the $R_f$ values. The reagents which were used for phytochemical screening are as the following;

For alkaloids detection, sprayed TLC plate with Dragendorff's reagent. Positive test after spraying this reagent is red-orange color (Farnsworth, 1966).

For terpenoid detection, sprayed TLC plate with Anisaldehyde-sulfuric acid and follow by heating in oven at 100-105 °C until maximal vitsulization. Positive test after spraying this reagent are colorful spots which occur varies on the compound; red (terpene), green (steroid), blue (phenol) and grey (sugar) (Merck, 1980).

For coumarin detection, sprayed TLC plate with 10% NaOH. When the plate dried, exposed the plate under UV light at wavelength 365 nm. If coumarin is present, a yellow-green fluorescence will be appeared (Farnsworth, 1966).

The position of a substance zone (spot) in a thin layer chromatogram can be described as Retardation Factor (Rf).

$$Rf = \frac{\text{distance of the substance zone from the starting line (cm)}}{\text{distance of the solvent front from the starting line(cm)}}$$

(Hahn-Deinstrop, 1997)

4.2.2 HPLC

Prepared 10 mg of samples in methanol (HPLC grade). The HPLC analysis is undertaken on Agilent 1100 series. Ultraviolet (UV) spectra were determined on Agilent 1100 series UV photodiode array detector 230 nm wave length at Scientific Instrumentation Center, Faculty of Science, Kasetsart University.

# RESULTS AND DISCUSSION

This study are divided into two parts which consisted of the study on morphological characters of *G. pentaphylla*, *G. parva* and *G. mauritiana* and study on chemical characters by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC).

## 1. Morphological Character

### 1.1 Habit

Thai *Glycosmis* species are usually shrub to small tree. The stems are straight and branching, unarmed. In this study divided into two groups shrub (0.5-1.0 m) and small tree (taller than 1.0-2.0 m).

The habit of shrub are seen in *G. pentaphylla* population 5 (50 cm), population 6 (80 cm), population 10 (80 cm) and population 13 (80 cm); all population of *G. parva*, population 1 (70 cm), population 2 (50 cm), population 3 (50 cm) and all population of *G. mauritiana*, population 1 (80 cm) and population 2 (70 cm) (Figure 2A, C and D).

Small trees are seen in *G. pentaphylla* population 1 (1.0 m), population 2 (1.2 m), population 3 (1.0 cm), population 4 (1.8 m), population 7 (1.0 m), population 8 (2.0 m), population 9 (1.2 m), population 11 (1. 5 m), population 12 (1.25 m) and population 14 (1.25 m) (Figure 2B).

### 1.2 Bark

The bark of *G. pentaphylla*, *G. parva* and *G. mauritiana* is usually smooth sometime lenticillate in old plant. The color of outer bark is reddish brown. Inner bark is white yellow sometime aromatic.

1.3 Leaves

The leaves are unifoliolate and pinnately compound, alternate arranged (sometime subopposite in apex of branch). The most of them are pinnately compound leaves but unifoliolate leaf are all population of *G parva* and sometime in *G. pentaphylla* population 8 and *G. mauritiana* population 1.

1.4 Leaflet

Leaves varies in number of leaflets, sizes and shapes can be usually seen in *G. pentaphylla*. Most of them have 5 leaflets i.e. population 3, 9, 10, 12 and population 14. Population 1, 8 and 13 have 3-5 leaflets. Population 5, 6, 7, and 11 have 7 leaflets but population 2 and 4 have 5 and 7 leaflet (Figure 3).

The blade is narrowly elliptic in all population of *G. parva* and population 2, 4, 6, 7, 10 and11 of *G. pentaphylla*), elliptic (*G. pentaphylla* population 3, 5, 9 and 12 and *G. mauritiana* population 2) and ovate (*G. pentaphylla* population 1, 8, 13 and 14 and G. *mauritiana* population 1) (Table 1).

The apex is obtuse, acute or attenuate. The margin is usually entire in *G. parva* and *G. mauritiana*, undulate or serrate in *G. pentaphylla* (rarely entire). The base is cuneate and oblique. The texture is papery, glabrous both surface sometime covered with dense fine-woolly rust-colored when young. The leaves are commonly dark green (rarely light green).

The petiole is always present long in pinntely population 1.0-4(5) cm in length, some populations can be long up to 5 cm (*G. pentaphylla* population 4), slender or cyclindric, glabrous. The petiole of *G. parva* is usually short 0.2-0.5 cm, glabrous. The petiolues of pinnately population are short ca 0.5 cm terete and glabrous.

The midrib are flat or raised above, raised beneath. The lateral veins are 6-12 pairs, flat above, raised beneath, arching and looping near margin. The tertiary veins reticulate, scalariform-reticulate prominent beneath.

1.5 Inflorescences

The solitary flower has been seen in *G. parva* of all populations and *G. mauritiana* population 1. The inflorescences of *G. pentaphylla* and *G. mauritiana* population 2 are panicle, 2.0-5.0 cm long in *G. pentaphylla* and 0.5-1.5 cm long in *G. mauritiana*.

The positions of inflorescences are usually both axillary and terminal position but some populations are only axillary (*G. pentaphylla* population 6) or terminal (*G. pentaphylla* population 13) (Figure 4).

The young panicles are still covered with dense fine-woolly rust-colored.

1.6 Flowers

The flowers are bisexual, more than 5 flowers per panicle, some species less than this (3-5 flower). They are globose or elliptic in bud; size, usually 0.5-1.5 cm long, white.

Number of sepal is usually 5, equal to subequal, imbricate, inner surface glabrous, while the outer surface are puberulous; ca 0.2 mm long. The petal is usually 5, basally connate, equal to subequal, imbricate, glabrous both surface (*G. parva* and *G. pentaphylla*) , puberulent outer surface and glabrous inner surface (*G. mauritiana*), white sometime pellucid dot present. The petal is caducous when flower mature.

The stamens are 10, arrange into 2 sets, the 1st set (outer set) has longer filament. The filaments are slender, 0.2-0.5 cm long, with glabrous sometimes puberulent (*G. mauritiana*). The anthers are 2 cells, elliptic, all are introse, the filament.

The ovary is superior, 0.3-0.5 cm long, 1-2 locular and with 1 ovule. The shape of the ovary is ovoid, globose, subglobose or ellipsoid. The texture of the ovary is glabrous in *G. parva* and *G. pentaphylla* and puberulent in *G. mauritiana*. The ovary is subtended by the annular disk (Figure 5).

For this study, the petal and stamen are not found in 4 populations, i.e. *G. parva* population 1, 2 and *G. mauritiana* population 1, 2.

1.7 Fruit

The fruits are berry sometimes supported by more or less persistent calyx or annular disk. The shape of fruits is globose, ovate; size is 0.5-1.5 cm long. The apex is apiculate stigma, persistent. The color of fruits is green, turning dark green, pink or dark pink (salmon red) when ripe. The surface is glabrous sometime colliculate with oil gland (*G. mauritiana*) (Figure 6). The seed are 1-2 seeds per fruit.

1.8 Ecology

The ecological range of *Glycosmis* in this study is widely from open area, dry evergreen forest and tropical evergreen rain forest. Most species occur in open area, dry evergreen forest near stream or waterfall. Some species are found on lime stone hill i.e. *G. pentaphylla* population 13 and all populations of *G. mauritiana*.


**Figure 2** Habit of *Glycosmis*: A. *G. pentaphylla* (shrub); B. *G. pentaphylla* (small tree); C. *G. parva* (shrub); D. *G. mauritiana* (shrub)

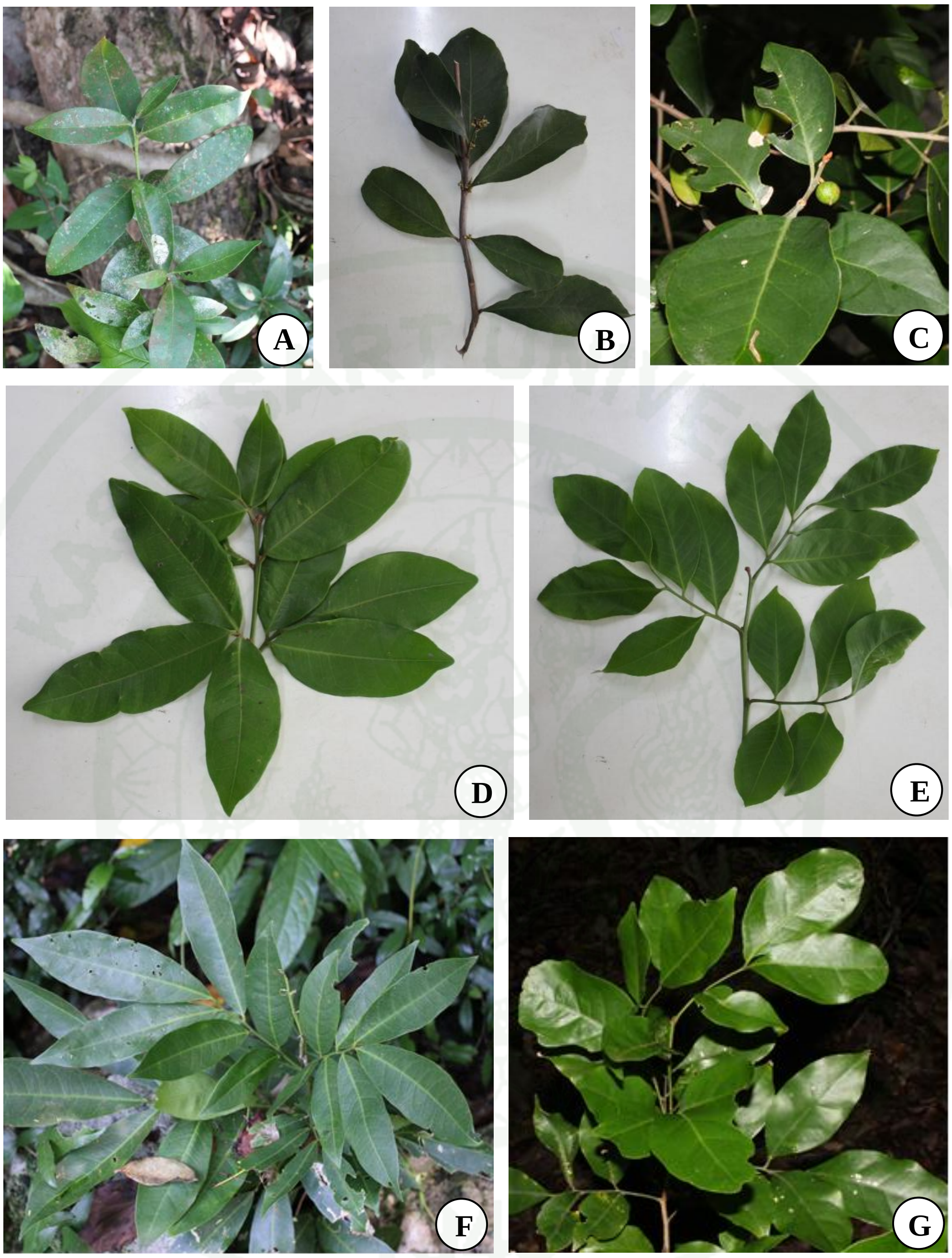



**Figure 3** Leaves of *Glycosmis*: A. *G. parva* (unifoliolate); B. *G. pentaphylla* (unifoliolate); C. *G. mauritiana* (unifoliolate); D. *G. pentaphylla*. (trifoliolate); E. *G. pentaphylla* (5-foliolate); F. *G. pentaphylla* (7-foliolate); G. *G. mauritiana* (trifoliolate)

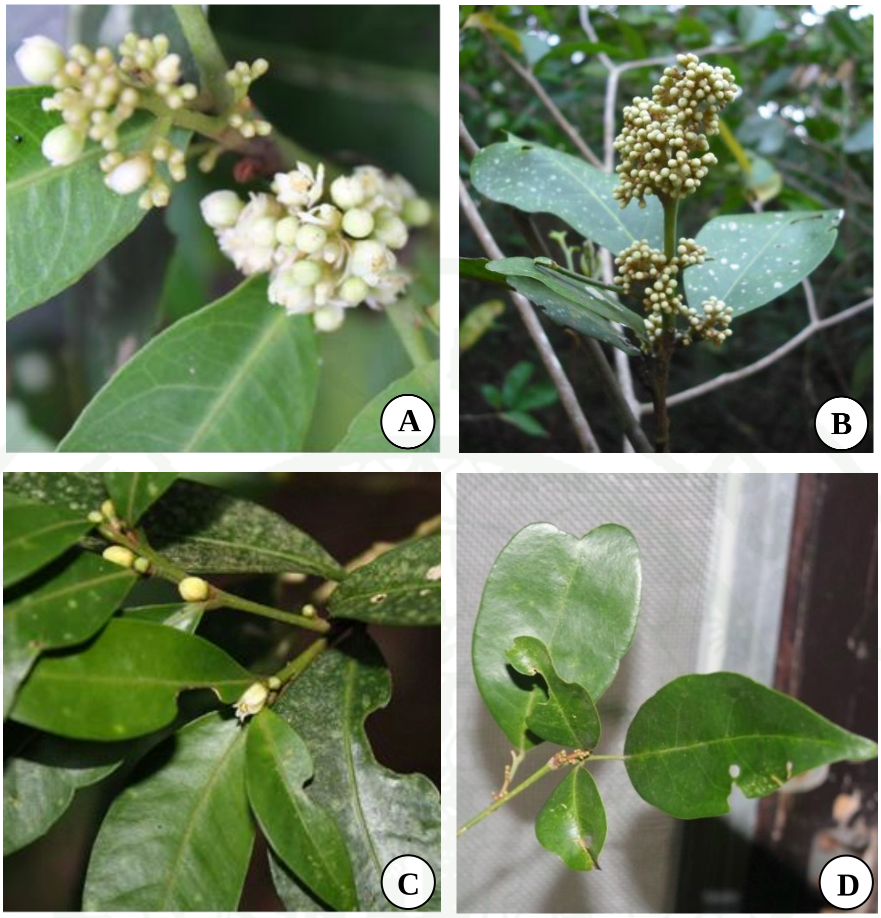



**Figure 4** Inflorescence of *Glycosmis*: A. *G. pentaphylla* (paniculate, axillary and terminal position); B. *G. pentaphylla* (paniculate, terminal position); C. *G. parva* (solitary, axillary and terminal position); D. *G. mauritiana* (paniculate, axillary and terminal position)

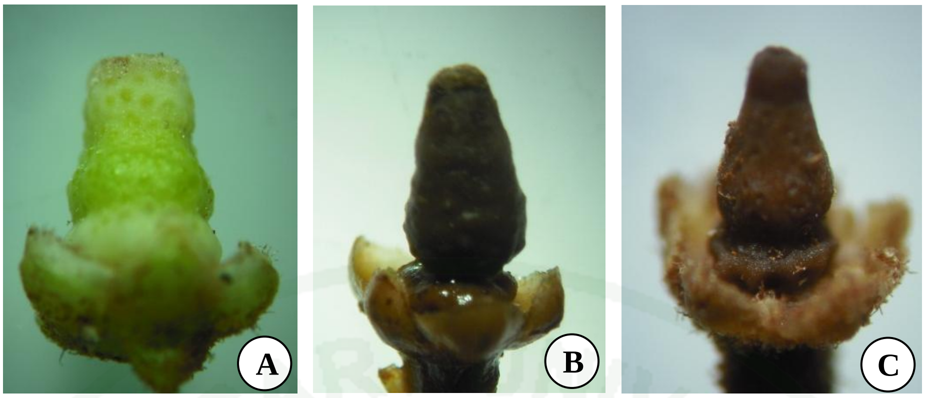



**Figure 5**  Ovary of *Glycosmis*: A. *G. pentaphylla*; B. *G. parva*; C. *G. mauritiana*

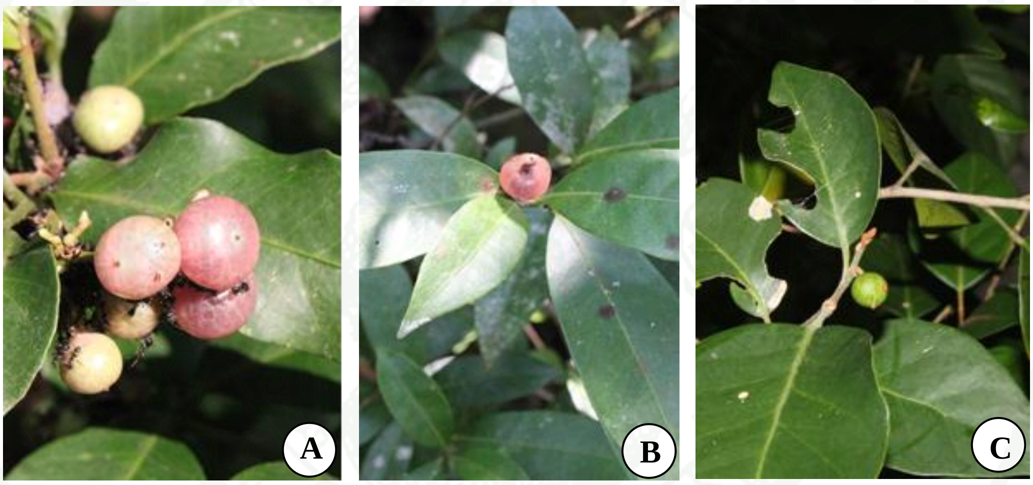



**Figure 6**  Fruit of *Glycosmis*: A. *G. pentaphylla*; B. *G. parva*; C. *G. mauritiana*

**Table 9** Some morphological characters of *Glycosmis*

| Species /population | Habit | Leaves type | Leaflet No. | Leaflet shape | Voucher /locality |
|---|---|---|---|---|---|
| *G. pentaphylla* population 1 | small tree 1.0 m | pinnately compound | 3-5 | ovate | W. Aiyakool 48 Trat |
| *G. pentaphylla* population 2 | small tree 1.2 m | pinnately compound | (5) 7 | narrowly elliptic | W. Aiyakool 42 Chanthaburi |
| *G. pentaphylla* population 3 | small tree 1.0 m | pinnately compound | 5 | elliptic | W. Aiyakool 46 Rayong |
| *G. pentaphylla* population 4 | small tree 1.8 m | cinnately compound | (5) 7 | narrowly elliptic | W. Aiyakool 33 Chon Buri |
| *G. pentaphylla* population 5 | shrub 50 cm | pinnately compound | 7 | elliptic | W. Aiyakool 40 Nakhon Nayok |
| *G. pentaphylla* population 6 | shrub 80 cm | pinnately compound | 7 | narrowly elliptic | W. Aiyakool 43 Saraburi |
| *G. pentaphylla* population 7 | small tree 1.0 m | pinnately compound | 7 | narrowly elliptic | W. Aiyakool 44 Nakhon Ratchasima |
| *G. pentaphylla* population 8 | small tree 2.0 m | pinnately compound | (1) 3-5 | ovate | W. Aiyakool 49 Bangkok |
| *G. pentaphylla* population 9 | small tree 1.2 m | pinnately compound | 5 | elliptic | W. Aiyakool 35 Phangnga |
| *G. pentaphylla* population 10 | shrub 80 cm | pinnately compound | 5 | narrowly elliptic | W. Aiyakool 36 Trang |
| *G. pentaphylla* population 11 | small tree 1.5 m | pinnately compound | 7 | narrowly elliptic | W. Aiyakool 39 Krabi |
| *G. pentaphylla* population 12 | small tree 1.25 m | pinnately compound | 5 | elliptic | W. Aiyakool 38 Nakhon Si Thammarat |

**Table 9** (Continued)

| Species /population | Habit | Leaves type | Leaflet No. | Leaflet shape | Voucher /locality |
|---|---|---|---|---|---|
| *G. pentaphylla* population 13 | shrub 80 cm | pinnately compound | 3-5 | ovate | W. Aiyakool 37 Satul |
| *G. pentaphylla* population 14 | small tree 1.25 m | pinnately compound | 5 | ovate | W. Aiyakool 34 Songkla |
| *G. parva* population 1 | shrub 70 cm | unifoliolate | 1 | narrowly elliptic | W. Aiyakool 45 Haew Narok Waterfall, |
| *G. parva* population 2 | shrub 50 cm | unifoliolate | 1 | narrowly elliptic | W. Aiyakool 47 Rayong |
| *G. parva* population 3 | shrub 50 cm | unifoliolate | 1 | narrowly elliptic | W. Aiyakool 41 Sakaerat |
| *G. mauritiana* population 1 | shrub 80 cm | pinnately compound | (1) 3 | ovate | W. Aiyakool 50 Prachuap Khiri Khan |
| *G. mauritiana* population 2 | shrub 70 cm | Pinnately Compound | 3 | elliptic | W. Aiyakool 51 Prachuap Khiri Khan |

1.9 Key to species and population descriptive

**Glycosmis** Corrêa, Ann. Mus. Natl. Hist. Nat. 6: 384. 1805; Craib, Fl. Siam. Enum. 2: 222. 1934; Guillaumin in H. Humbert, Fl. Indo-Chine 5: 619. 1946; B. C. Stone in Whitmore Tree Fl. Malaya 1: 380. 1972; B. C. Stone, Proc. Acad. Nat. Sci. Philad. 137: 24.

Shrub or small tree, unarmed, evergreen, aromatic, young shoot covered with dense fine-woolly rust-colored. *Leaves* alternate odd-pinnate or unifoliolate.

*Inflorescence* terminal and/or axillary, paniculate or reduced to 1 flower. *Flower* bisexual, globose to ellipsoid in bud, sepals 5, basally connate; petals 5, imbricate in bud; stamens 10, distinct, alternately unequal in length; filament straight; disk annular shaped; ovary superior, 1-2 loculed; ovules 1per locule; style to nearly as long as ovary, persistent in fruit. *Fruit* a berry, with mucilaginous pulp or dry, without pulp vesicles; endocarp membranous. *Seed* 2 per fruit, with membranous seed coat; endosperm lacking; embryo straight; cotyledons elliptic, plano-convex.

Key to species of tree groups of *Glycosmis* in Thailand.

1. Ovary grabrous
   2. Leaves unifoliolate …………….…………………………….....…....….. *G. parva*
   2. Leaves pinnately compound ………………………….………… *G. pentaphylla*
1. Ovary puberulous ……………………………………………….….. *G. mauritiana*

**1. Glycosmis pentaphylla** (Retz.) DC., Prodr. 1: 538. 1824; Craib, Fl. Siam. Enum. 2: 224. 1934; Guillaumin in H. Humbert, Fl. Indo-Chine 5: 624. 1946; B. C. Stone in Whitmore, Tree Fl. Malaya 1: 382. 1972. — *Limonia pentaphylla* Retz., Obs. Bot. 5: 24. 1788. — *Glycosmis arborea* DC., Prodr. 1: 538. 1824. — *G. rupestris* Ridl., J. Straits. Br. Roy. Asiat. Soc. 59: 81. 1911. — *G. longipes* Tanaka, Bull. Mus. Natl. Hist. Nat. 2. 2. 159. 1930.

Shrub or small tree 0.5-2 m high. *Bark* smooth, reddish brown, inner bark white yellow. *Leaves* (1) 3 -5 (7) –foliolate, 10-30 cm long; petiole 1.0-5.0 cm; *leaflet* ovate to elliptic, glabrous on both surface, papery, dark green, base crenate to slightly oblique, margin serrate or undulate, apex acute-round acute, 6.0-20 by 2.0-6.0 cm; petiolules terete ca. 0.5 cm long, midrib flat or raised above, raised beneath, lateral veins 6-12 pairs, flat or raised above, raised beneath, arching and looping near margin, tertiary veins reticulate, prominent beneath. *Inflorescences* axillary and/or terminal, 2.0-15 cm long, paniculate, covered with dense fine-woolly rust-colored.

*Flower* bisexual, globose to ellipsoid in bud, 0.5-1.5 mm long; sepals 5, basally connate, broadly ovate, puberulous outside, glabrous inside, less than 1 mm long; petal 5, white, ovate, glabrous both surface, 0.4-1.2 mm by 0.3-1.0 mm, caducous; stamens 10, distinct, alternately unequal in length; filaments straight, glabrous, 0.2-0.8 mm long; disk annular shaped; ovary superior, ovoid, glabrous, style as long as ovary, 0.3-0.8 mm long, persistent in fruit, stigma slightly expanded. *Fruit* berry, green, pink or dark pink (salmon red), globose, glabrous, 0.5-1.5 cm long; seed 2 per fruit.

Thailand.— NORTHERN: Mae Hong Son, Chiang Mai, Chiang Rai, Lamphun, Lampang, Phrae, Tak, Phitsanulok, Sukhothai, Khumpeng Phet; NORTH-EASTERN: Phetchabun, Loei, Chaiya Phum, Sakon Nakhon; EASTERN: Nakhon Ratchasima, Surin, Buri Ram; SOUTH-WESTERN: Kanchanaburi, Uthaithani, Ratchaburi, Prachuap Khiri Khan; CENTRAL: Saraburi, Nakhon Nayok; SOUTH-EASTERN: Prachin Buri, Chachoengsao, Chonburi, Rayong, Chantaburi, Trat; PENINSULAR: Suratthani, Pangnga, Trang, Satun, Yala, Pattani.

Ecology.— Mixed deciduous or Dry evergreen forest or open area; altitude 10–500 m.

Vernacular.— Khoei tai (เขยตาย), Nam khao (น้ำข้าว), Som chuen (ส้มชื่น)

**Glycosmis pentaphylla population 1** (Figure 7, Appendix Figure 3)

Small tree 1.0 m high. *Leaves* 15-20 cm long; petiole cyclindric 1.5-2.0 cm long ; *leaflet* 3-5, ovate, base crenate to slightly oblique, apex acute, 8-14 cm by 3-6 cm, lateral veins 8-10 pairs. *Inflorescence* axillary and terminal, 5-15 cm long.

*Flower* globose or elliptic in bud, 0.8-1.0 mm long, sepal ca. 0.2 mm long; petal 0.6-0.8 mm long and 0.4-0.6 mm wide, filament ca. 0.5 mm; ovary 0.3-0.6 mm long. *Fruit* 1.0-1.2 cm long.

Locality. – Trat Agroforestry Research and Training Station, Trat.

Ecology. – Open area, dry evergreen forest.

Voucher specimens. – *W. Aiyakool 48* (29 Jan 2012)

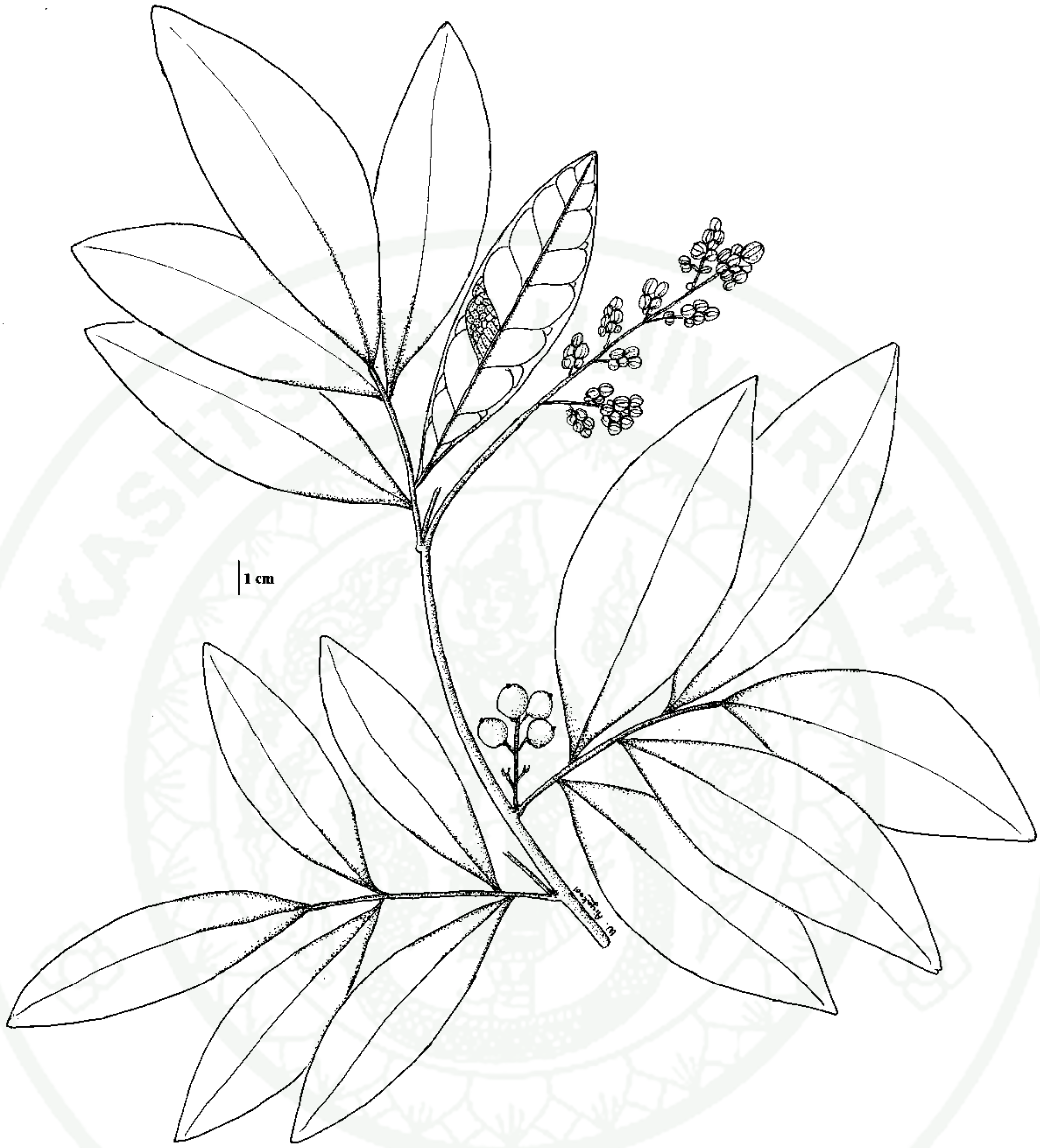



**Figure 7** *G. pentaphylla* population 1 (Trat; Trat Agroforestry Research and Training Station; W. Aiyakool 48), drawn by W. Aiyakool

**<u>Glycosmis</u> <u>pentapyhylla</u> population 2** (Figure 8, Appendix Figure 4)

Small tree 1.2 m. *Leaves* 15-25 cm long; petiole 1.5-3 cm long; *leaflet* (5) 7, narrowly elliptic, base oblique, apex round-acute, 7-15 cm by 3-5 cm, lateral veins 9-12 pairs. *Inflorescence* axillary and terminal, 2-10 cm long.

*Flower* globose in bud, 0.5-0.8 mm long; petal 0.4-0.6 mm by 0.3-0.5 mm; stamen filament ca. 0.3 mm long; ovary 0.3-0.5 mm long. *Fruit* 0.8-1.0 cm long.

Locality. – Krating waterfall, Chanthaburi.

Ecology. – Dry evergreen forest; near waterfall.

Voucher specimens. – *W. Aiyakool 42* (10 Dec 2011)

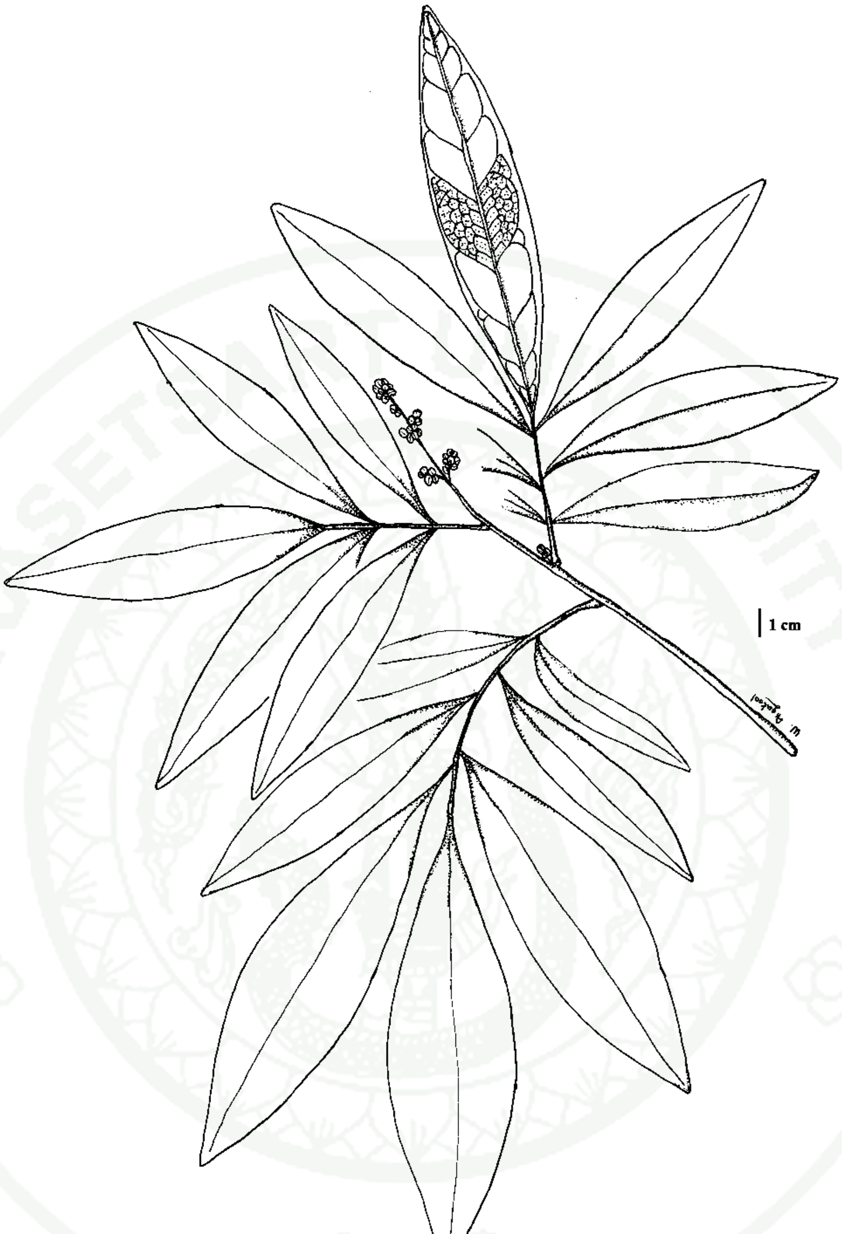



**Figure 8** *G. pentaphylla* population 2 (Chanthaburi; Krating Waterfall; W. Aiyakool 42), drawn by W. Aiyakool

**<u>Glycosmis</u> <u>pentaphylla</u> population 3** (Figure 9, Appendix Figure 5)

Small tree 1.0 m. *Leaves* 20-25 cm long; petiole 2-4 cm long; *leaflet* 5, elliptic, base cuneate to oblique, margin undulate, apex round-acute, 10-20 cm by 4.5-6 cm, lateral veins 6-10 pairs. *Inflorescence* axillary and terminal, 2-4 cm long.

*Flower* globose in bud, 0.8-1.0 mm long; petal 0.6-0.8 mm by 0.4-0.7 mm, caducous; stamen filament ca. 0.5 mm; ovary 0.3-0.5 mm long. *Fruit* green, 0.5-1.0 cm long.

Locality. – Khao Chamao, Rayong.

Ecology. – Open area; dry evergreen forest; near waterfall.

Voucher specimens. – *W. Aiyakool 46* (28 Jan 2012)


**Figure 9** *G. pentaphylla* population 3 (Rayong; Khao Chamao; W. Aiyakool 46), drawn by W. Aiyakool

**<u>Glycosmis</u> <u>pentaphylla</u> population 4** (Figure 10, Appendix Figure 6)

Small tree 1.8 m high. *Leaves* 18-30 cm long, alternate or subopposite; petiole cyclindric 2-5 cm long; *leaflet* (5) 7, narrowly elliptic, 7-15 cm by 3-5 cm, lateral veins 6-10 pairs. *Inflorescence* axillary and terminal, 5-12 cm long.

*Flower* globose in bud, 1.0-1.2 mm long; petal 0.8-1.0 mm by 0.5-0.8 mm; stamen filament ca. 0.5 mm; ovary 0.4-0.6 mm long. *Fruit* 0.5-0.8 cm long.

Locality. – Kasetsart University, Sriracha Campus, Chon Buri.

Ecology. – Common in open area, dry evergreen forest; near coast.

Voucher specimens. – *W. Aiyakool 33* (25 Nov 2010)


**Figure 10** *G. pentaphylla* population 4 (Chonburi; Kasetsart University Siracha Campus; W. Aiyakool 33), drawn by W. Aiyakool

**<u>Glycosmis</u> <u>pentaphylla</u> population 5** (Figure 11, Appendix Figure 7)

Shrub 50 cm high. *Leaves* 16-20 cm long; petiole 1.2-2.0 cm long; *leaflet* 7, elliptic, margin undulate, apex acute, 8-12 cm long by d 3-4 cm, lateral veins 6-8 pairs. *Inflorescence* axillary and terminal, 3-5 cm long.

*Flower* globose in bud, 0.8-1.0 mm; petal 0.6-0.8 mm by 0.4-0.6 mm; filament ca. 0.5 mm; ovary 0.4-0.5 mm long. *Fruit* 0.8-1.0 cm long.

Locality. – Khao Cha ngok, Nakhon Nayok.

Ecology. – Open area, dry evergreen forest; near waterfall.

Voucher specimens. – *W. Aiyakool 40* (8 July 2011)


**Figure 11** *G. pentaphylla* population 5 (Nakhon Nayok; Khao Cha-ngok; W. Aiyakool 40), drawn by W. Aiyakool

**<u>Glycosmis</u> <u>pentaphylla</u> population 6** (Figure 12, Appendix Figure 8)

Shrub 80 cm high. *Leaves* 18-25 cm long; petiole 2.0-3.5 cm long; leaflet 7, narrowly elliptic, margin undulate, 15-20 cm by 4-6 cm, lateral veins 8-10 pairs. *Inflorescence* axillary, 2-5 cm long.

*Flower* globose in bud, 0.8-1.2 mm long; petal 0.5-0.8 mm by 0.4-0.6 mm; stamen filament ca. 0.4 mm; ovary 0.3-0.5 mm. *Fruit* 0.8-1.2 cm long.

Locality. – Sam Lan Waterfall, Saraburi.

Ecology. – Open area, dry evergreen forest; near waterfall.

Voucher specimens. – *W. Aiyakool 43* (15 Dec 2011)


**Figure 12** *G. pentaphylla* population 6 (Saraburi; Sam Lan Waterfall; W. Aiyakool 43), drawn by W. Aiyakool

**Glycosmis pentaphylla population 7** (Figure 13, Appendix Figure 9)

Shrub 80 cm high. *Leaves* 15-17 cm long; petiole 2.0-3.5 cm long; *leaflet* 7, narrowly elliptic, 6.0-12.0 cm by 2.0-4.0 cm, lateral veins 8-12 pairs. *Inflorescence* axillary and terminal, 2.5-5.0 cm long.

*Flower* globose in bud, 1.2-1.5 mm long; petal 0.6-1.0 mm by 0.5-0.8 mm; stamen, filament ca. 0.4 mm; ovary 0.4-0.6 mm long. *Fruit* green, 0.8-1.2 cm long.

Locality. – Haew Narok Waterfall, Nakhon Ratchasima.

Ecology. – Riverside, open area, dry evergreen forest; near waterfall.

Voucher specimens. – *W. Aiyakool 44* (7 Jan 2012)


**Figure 13** *G. pentaphylla* population 7 (Nakhon Ratchasima; Haew Narok Waterfall; W. Aiyakool 44), drawn by W. Aiyakool

**<u>Glycosmis</u> <u>pentaphylla</u> population 8** (Figure 14, Appendix Figure 10)

Small tree 2.0 m high. *Leaves* 12-18 cm long; petiole 1.0-1.5 cm long; *leaflet* (1) 3-5, ovate, 8-14 cm by 3-6 cm, lateral veins 10-12 pairs. *Inflorescence* axillary and terminal, 5-12 cm long.

*Flower* globose or elliptic in bud, 1.0-1.5 mm long; petal 0.8-1.2 mm by 0.6-1.0 mm; stamen filament ca. 0.8 mm; ovary 0.5-0.8 mm long. *Fruit* 1.0-1.5 cm.

Locality. – Bangkok.

Ecology. – Cultivated.

Voucher specimens. – *W. Aiyakool 49* (29 June 2012)

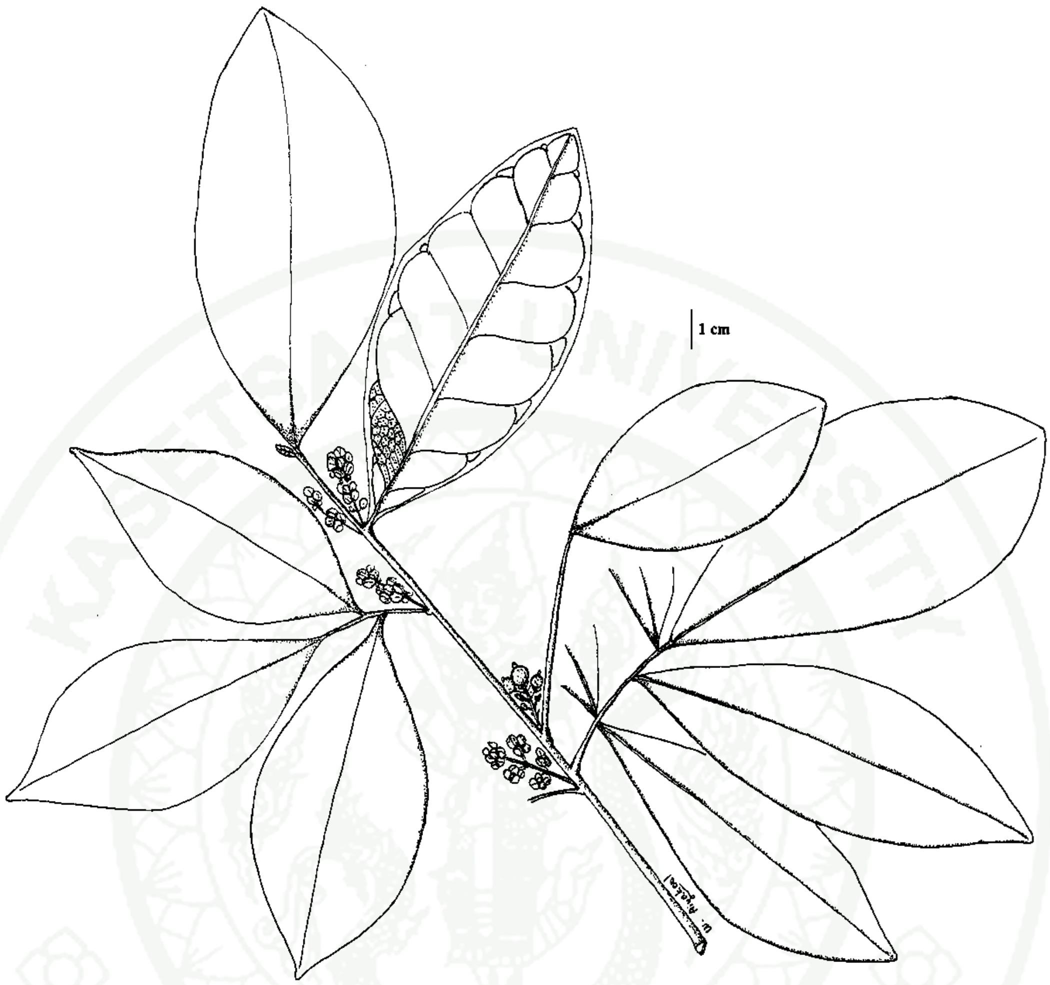



**Figure 14** *G. pentaphylla* population 8 (Bangkok; W. Aiyakool 49), drawn by W. Aiyakool

**<u>Glycosmis</u> <u>pentaphylla</u> population 9** (Figure 15, Appendix Figure 11)

Small tree 1.2 m high. *Leaves* 18-22 cm long; petiole 2-2.5 cm long; *leaflet* 5, elliptic, 10-20 cm by 4-6 cm, lateral veins 8-12 pairs. *Inflorescence* axillary and terminal, 4-12 cm long.

*Flower* globose in bud, 1.2-1.5 mm long; petal 0.8-1.2 mm by 0.5-0.8 mm; stamen filament ca. 0.5 mm; ovary 0.4-0.8 mm long. *Fruit* 0.8-1.0 cm long.

Locality. – Sa Nang Manora Waterfall, Phangnga.

Ecology. – Evergreen forest, near waterfall.

Voucher specimens. – *W. Aiyakool 35* (5 May 2011)


**Figure 15** *G. pentaphylla* population 9 (Phangnga; Sa Nang Manora Waterfall; W. Aiyakool 35), drawn by W. Aiyakool

**<u>Glycosmis</u> <u>pentaphylla</u> population 10** (Figure 16, Appendix Figure 12)

Shrub 80 cm high. *Leaves* 15-20 cm long; petiole 1.5-2.0 cm long; *leaflet* 5, narrowly elliptic, 6-15 cm by 2-5 cm, lateral veins 8-12 pairs. *Inflorescence* axillary and terminal, 2-8 cm long.

*Flower* globose in bud, 1.2-1.5 mm long; petal 0.8-1.2 mm by 0.7-0.9 mm; stamen, filament ca. 0.5 mm; ovary 0.4-0.8 mm long. *Fruit* 0.5-0.8 cm.

Locality. – Thung Khai Botanical Garden , Trang.

Ecology. – Evergreen forest.

Voucher specimens. – *W. Aiyakool 36* (6 May 2011)


**Figure 16** *G. pentaphylla* population 10 (Trang; Thung Khai Batanical Garden; W. Aiyakool 36), drawn by W. Aiyakool

**<u>Glycosmis</u> <u>pentaphylla</u> population 11** (Figure 17, Appendix Figure 13)

Small tree 1.5 m high. *Leaves* 18-25 cm long; petiole 2.0-4.0 cm long; *leaflet* 7, narrowly elliptic, 8-12 cm by 4-5 cm, lateral veins 8-12 pairs. *Inflorescence* axillary and terminal, 2.5-4.0 cm long.

*Flower* globose in bud, 0.8-1.0 mm long; petal 0.5-0.8 mm by 0.4-0.6 mm; stamen filament ca. 0.4 mm; ovary 0.3-0.6 mm long. *Fruit* 0.8-1.2 cm long.

Locality. – Sa Morakot, Krabi.

Ecology. – Evergreen forest.

Voucher specimens. – *W. Aiyakool 39* (14 May 2011)


**Figure 17** *G. pentaphylla* population 11 (Krabi; Sa Morakot; W. Aiyakool 39), drawn by W. Aiyakool

**<u>Glycosmis</u> <u>pentaphylla</u> population 12** (Figure 18, Appendix Figure 14)

Small tree 1.25 m high. *Leaves* 10-15 cm long; petiole 1.0-2.5 cm long; *leaflet* 5, eliptic, 8-12 cm by 3-5 cm, lateral veins 10-12 pairs. *Inflorescence* axillary and terminal, 2-5 cm long.

*Flower* globose in bud, 0.5-1.0 mm long; petal 5 0.5-0.8 mm by 0.4-0.6 mm; stamen filament ca. 0.4 mm; ovary 0.4-0.6 mm long. *Fruit* 0.8-1.5 cm long.

Locality. – Karome Waterfall, Nakhon Si Thammarat.

Ecology. – Evergreen forest.

Voucher specimens. – *W. Aiyakool 38* (10 May 2011)

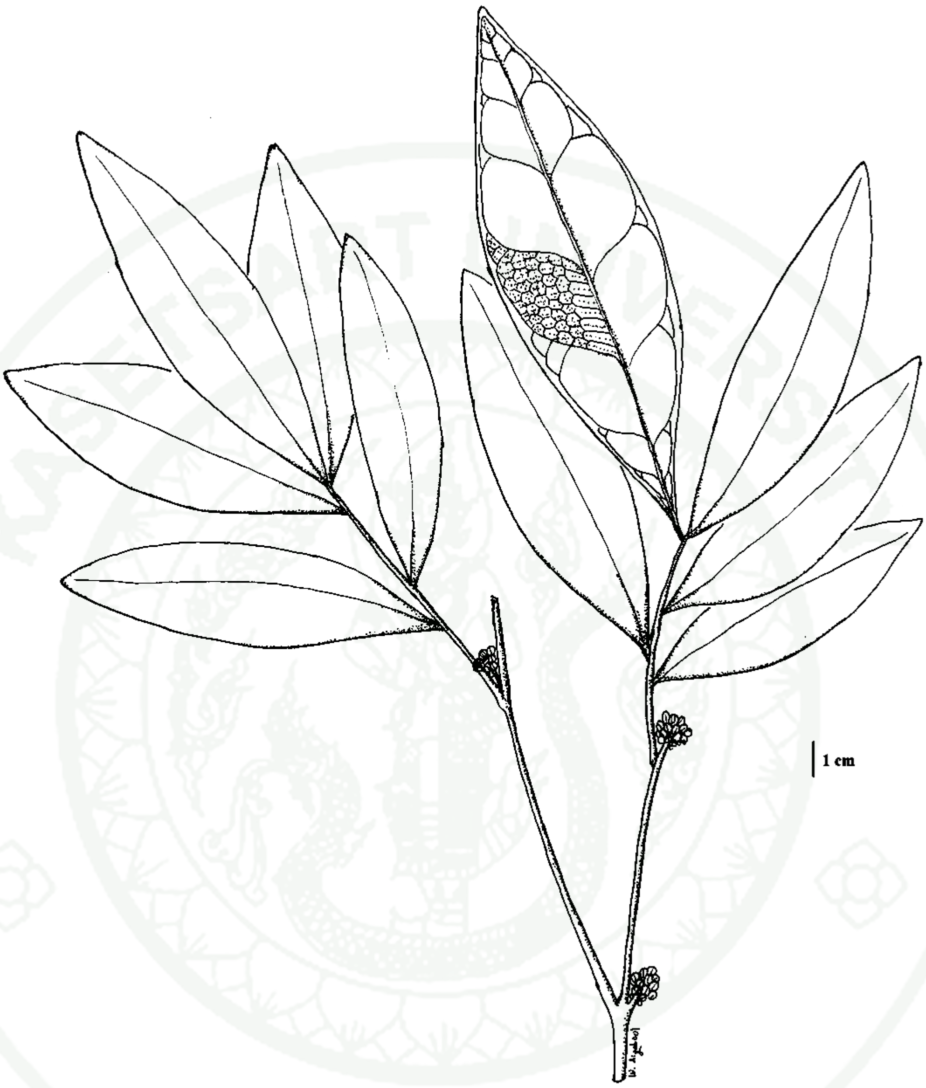



**Figure 18** *G. pentaphylla* population 12 (Nakhon Si Thammarat; Karome Waterfall; W. Aiyakool 38), drawn by W. Aiyakool

**<u>Glycosmis</u> <u>pentaphylla</u> population 13** (Figure 19, Appendix Figure 15)

Shrub 80 cm high. *Leaves* 12-18 cm long; petiole 1.0-2.0 cm long; *leaflet* 3-5, ovate, 8-14 cm by 3-6 cm wide, lateral veins 6-12 pairs. *Inflorescence* terminal, 2-5 cm long.

*Flower* globose in bud, 1.2 mm long; petal 0.8-1.0 mm by 0.5-0.6 mm; stamen filament ca. 0.5 mm; ovary 0.5-0.8 mm long. *Fruit* 0.5-1.2 cm long.

Locality. – Tarutao Island National Park, Satun.

Ecology. – Limestone hill.

Voucher specimens. – *W. Aiyakool 37* (8 May 2011)

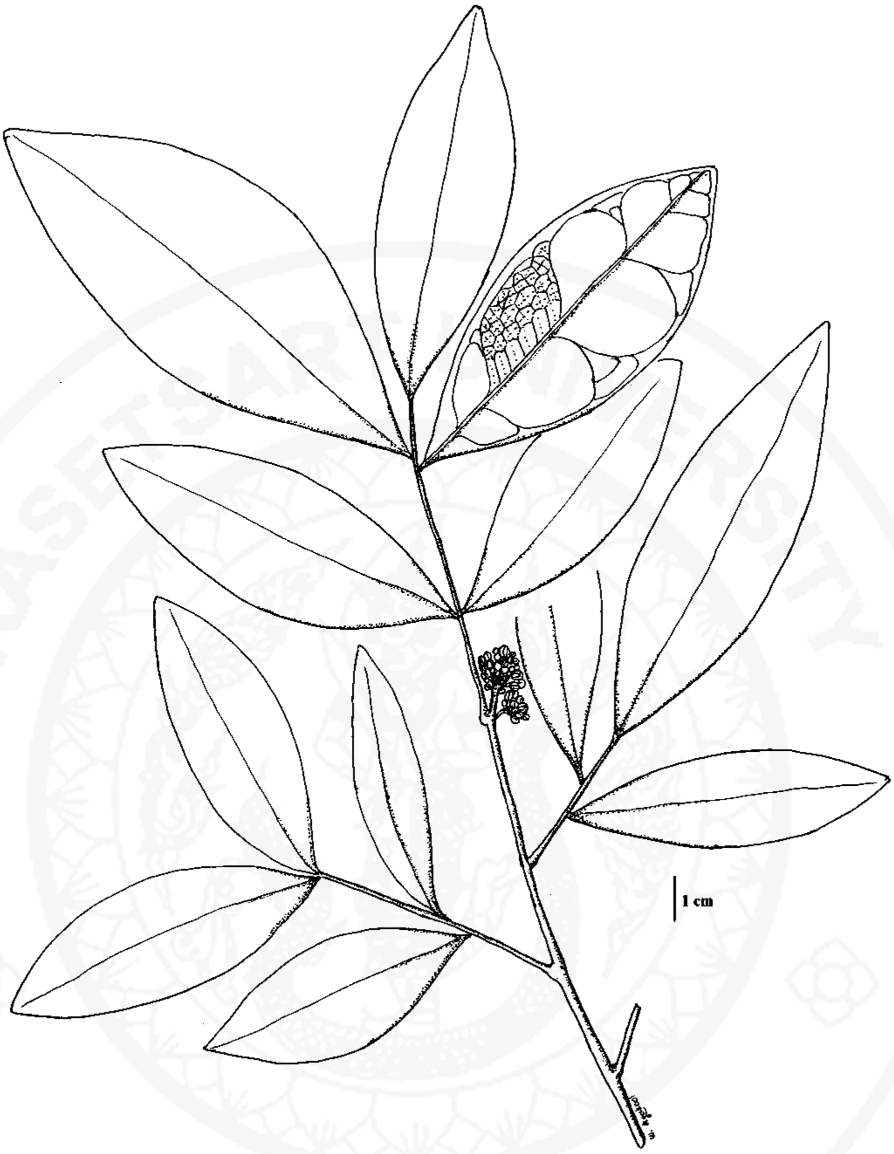



**Figure 19** *G. pentaphylla* population 13 (Satun; Tarutao Island; W. Aiyakool 37), drawn by W. Aiyakool

**Glycosmis pentaphylla population 14** (Figure 20, Appendix Figure 16)

Small tree 1.25 m high. *Leaves* 10-12 cm long; petiole 1.5-2.0 cm long; *leaflet* 5, ovate, 6-12 cm by 2-4 cm, lateral veins 8-12 pairs. *Inflorescence* axillary and terminal, 10-15 cm long.

*Flower* globose in bud, 1.0 mm; petal 0.8-1.0 mm by 0.6-0.8 mm; stamen filament ca. 0.2 mm; ovary 0.4-0.5 mm long. *Fruit* 0.8-1.0 cm long.

Locality. – Khao Noi, Songkhla.

Ecology. – Dry evergreen forest.

Voucher specimens. – *W. Aiyakool 34* (16 March 2011)


**Figure 20** *G. pentaphylla* population 14 (Songkhla; Khao Noi; W. Aiyakool 34), drawn by W. Aiyakool

**2. Glycosmis parva** Craib, Kew Bull. Misc. Inf. 337. 1926; Craib, Fl. Siam. Enum. 2: 223. 1934; Guillaumin in H. Humbert, Fl. Indo-Chine 5: 622. 1946; B. C. Stone, Proc. Acad. Nat. Sci. Philad. 137: 24. 1985.

Shrub 50-100 cm high, unarmed, young stem sometimes angular. *Leaves* unifoliolate; alternate sometime subopposite; petiole 0.2-0.5 cm; leaf blade narrowly elliptic, glabrous on both surface, papery, dark green, base cuneate, margin entire, apex obtuse, 4.0-12 by 10-3.5 cm; midrib raised above, raised beneath; lateral veins 6-12 pairs, flat above, raised beneath, arching and looping near margin, tertiary veins reticulate, prominent beneath.

*Flower* solitary, 2.0-5.0 mm, axillary and terminal, sepal 5, broadly ovate, glabrous both surface, less than 1 mm; petal 5, ovate, white, caducous, imbricate in bud, 1.0-4.0 mm; stamen 10, filament ca. 1.0 mm linear; ovary superior, ovoid, glabrous, 1.0-1.5 mm long; style to nearly as long as ovary, persistent in fruit. *Fruit* berry, green, pink when mature, globose to ovate, 0.7-1.0 by 0.5-0.9 cm; one seed per fruit.

Thailand.— EASTERN: Nakhon Ratchasima; SOUTH-WESTERN: Phetchaburi, Prachuap Khiri Khan; SOUTH-EASTERN: Prachin Buri, Chachoengsao, Chon buri, Rayong.

Ecology.— Dry evergreen forest; altitude 0–100 m.

**<u>Glycosmis</u> <u>parva</u> population 1** (Figure 21, Appendix Figure 17)

Shrub 70 cm high. *Leaves* unifoliolate; leaf blade narrowly elliptic, 1.5-3.5 cm by 5-12 cm, petiole ca. 0.2 cm long, lateral veins 6-10 pairs. *Ovary* ca. 1.0 mm long. *Fruit* berry, green, pink when mature, globose to ovate, 0.7 cm by 0.5 cm.

Locality. – Haew Narok Waterfall, Nakhon Ratchasima.

Ecology. – Common in open area; dry evergreen forest; near waterfall.

Voucher specimens. – *W. Aiyakool 45* (17 Jan 2012)

**<u>Glycosmis</u> <u>parva</u> population 2** (Figure 22, Appendix Figure 18)

Shrub 50 cm high. *Leaves* unifoliolate; leaf blade narrowly elliptic, 1.5-3.5 cm by 4.0-9.0 cm, petiole ca. 0.2 cm long, lateral veins 8-12 pairs. *Ovary* ca 1.2 mm long. *Fruit* berry, pink, globose to ovate, 1.0 cm long by 0.9 cm.

Locality. – Khao Chamao, Rayong.

Ecology. –Evergreen forest; near waterfall.

Voucher specimens. – *W. Aiyakool 47* (28 Jan 2012)


**Figure 21** *G. parva* population 1 (Nakhon Ratchasima; Haew Narok Waterfall; W. Aiyakool 45), drawn by W. Aiyakool


**Figure 22** *G. parva* population 2 (Rayong; Khao Chamao; W. Aiyakool 47), drawn by W. Aiyakool

**Glycosmis parva population 3** (Figure 23, Appendix Figure 19)

Shrub 50 cm high. *Leaves* unifoliolate; petiole ca 0.5 cm long; leaf blade narrowly elliptic, 1.0-3.0 cm by 4.0-8.0 cm, lateral veins 6-12 pairs. *Ovary* ca. 0.7 mm long. *Fruit* berry, pink, globose, 1.0 cm in diam.

Locality. – Sakaerat Environmental Research Station, Nakhon Ratchasima.

Ecology. – Common in open area, dry evergreen forest.

Voucher specimens. – *W. Aiyakool 41* (17 Nov 2011)


**Figure 23** *G. parva* population 3 (Nakhon Ratchasima; Sakaerat Environmental Research Station; W. Aiyakool 41), drawn by W. Aiyakool

**3. Glycosmis mauritiana** (Lam.) Tanaka, Bull. Soc. Bot. France 75: 708. 1928; B. C. Stone, Proc. Acad. Nat. Sci. Philad. 137: 13. 1985. — *Limonia mauritiana* Lam., Encycl. Meth. Bot. 3: 517. 1792.

Shrub to small tree 50-150 cm high, unarmed, evergreen, aromatic, young shoot covered with dense fine-woolly rust-colored. *Leaves* (1) 3-5 foliolate, alternate sometimes subopposite; petiole 2.0-5.0 cm; *leaflet* blade ovate to elliptic, glabrous on both surface, papery, dark green, base obtuse, margin entire, apex acute, 4.0-9.0 x 3.0-5.0 cm, midrib raised above, raised beneath, lateral veins 5-12 pairs, flat above, raised beneath, arching and looping near margin, tertiary veins reticulate, prominent beneath.

*Inflorescence* or solitary flower, axillary, 0.5-1.5 cm long, covered with dense fine-woolly rust-colored when young. *Flower* globose in bud, 2.0-2.5 mm long, 5 merous, sepal 5, broadly ovate, puberulous outside, glabrous inside, less than 1 mm; petal 5, white, ovate, glabrous always puberulent both surface, ca. 2.0 mm by 1.0 mm, caducous; stamen 10, filament ca. 1.0 mm, linear, puberulent, anther elliptic, ca. 0.5 mm; ovary superior, ovoid, puberulent, ca. 1.0 mm, style extremely short. *Fruit* berry, green, colliculate with oil gland, globose, 1.0-3.0 cm by 1.5-2.5 cm; seed 2 per fruit.

Thailand.— CENTRAL: Nakhon Nayok; SOUTH-WESTERN: Prachuap Khiri Khan; PENINSULAR: Suratthani, Trang, Pattatung.

Ecology.— Evergreen forest, Limestone hill; altitude 0-400m.

**<u>Glycosmis</u> <u>mauritiana</u> population 1** (Figure 24, Appendix Figure 20)

Shrub 80 cm high. *Leaves* 10-15 cm long; *leaflet* (1) 3; leaf blade 4.0-7.0 cm by 2.0-4.5 cm, lateral veins 5-6 pairs. *Inflorescence* racemose or solitary flower, axillary, 0.5-0.7 cm long. *Flower* ca. 2.5 mm long. *Fruit* 2.0-3.0 cm by 1.6-2.5 cm.

Locality. – Laem Sala, Prachuap Khiri Khan.

Ecology. – Open area, limestone hill.

Voucher specimens. – *W. Aiyakool 50* (15 Nov 2012)

**<u>Glycosmis</u> <u>mauritiana</u> population 2** (Figure 25, Appendix Figure 21)

Shrub 70 cm high. *Leaves* , 10-12 cm long; *leaflet* 3; leaf blade elliptic, 6.0-9.0 cm by 3.0-5.0 cm, lateral veins 8-12 pairs. *Inflorescence* or solitary flower, axillary and terminal, 0.5-1.5 cm long. *Flower* ca. 2.0 mm long. *Fruit* 1.4-2.0 cm by 1.0-1.4 cm.

Locality. – Trail to Praya Nakhon Cave, Prachuap Khiri Khan.

Ecology. – Open area, limestone hill.

Voucher specimens. – *W. Aiyakool 51* (15 Nov 2012)


**Figure 24** *G. mauritiana* population 1 (Prachuap Khiri Khan; Laem Sala; W. Aiyakool 50), drawn by W. Aiyakool

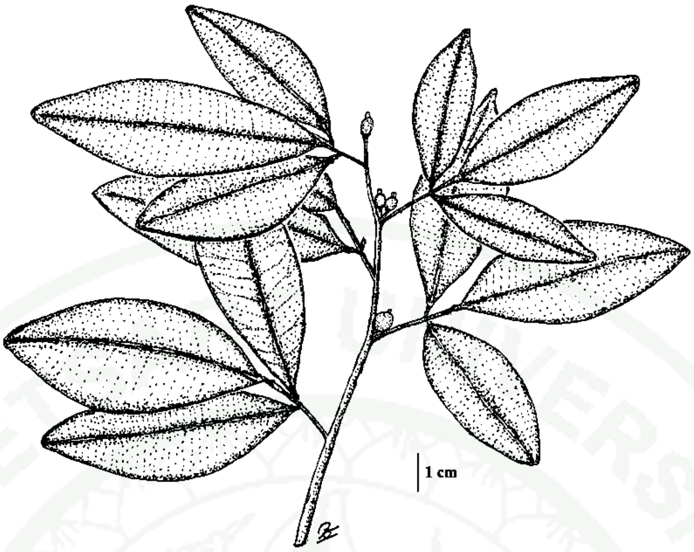



**Figure 25** *G. mauritiana* population 2 (Prachuap Khiri Khan; Trail to Praya Nakhon Cave; W. Aiyakool 51), drawn by W. Aiyakool

## 2. Phytochemical Character

### 2.1 Thin layer chromatography (TLC)

Phytochemical screening by using TLC technique, specific color reagents were sprayed on the TLC plates in order to detect alkaloids, terpenoids, coumarins and also to look for chemical profiles. The results of chemical detection by color reagent were shown in Table 10 and 11.

#### 2.1.1 Screening for alkaloids

After spraying Dragendorff's reagent onto the TLC plate of stem bark and leaf extracts from all populations of *G. parva*, *G. mauritiana* and *G. pentaphylla*. The results were shown in Table 10 and 11 and Figure 26, 27 and 28.

Stem bark and leaves extracts of *G. pentaphylla* collecting from central and eastern of Thailand gave positive test only populations 6 (Saraburi) and 7 (Nakhon Ratchasima) at Rf value 0.38. In southern group, the extracts from stem bark of population 4 (Nakhon Si Thammarat) and leaves of population 5 (Satun), alkaloids could not be detected while the other populations gave positive result at Rf value 0.16. The leaves extract of population 4 from Nakhon Si Thammarat, alkaloid could be detected at Rf value 0.16 and 0.20.

The results of alkaloids from stem bark extract of *G. parva* were detected as orange color at Rf values 0.47 and 0.57 but only 0.57 for population 1 (Nakhon Ratchasima : Haew Narok Waterfall) while leaves extracts of all populations showed positive test at same Rf value 0.14. In stem bark extract of *G. mauritiana* alkaloid was detected at Rf values 0.30 and in leaves extracts at at Rf values 0.03 and 0.06. So, this result showed similarity of alkaloids patterns in same organ (stem bark and leaves) from different population collected in the south.

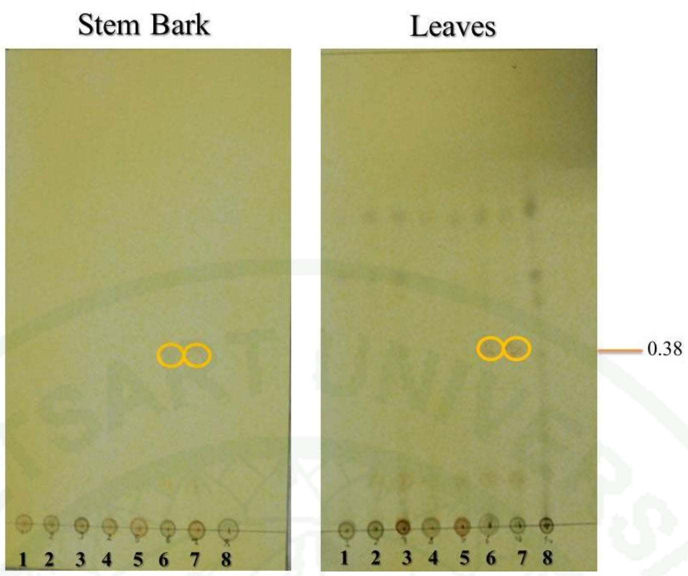



**Figure 26** TLC patterns of stem bark and leaves extracts of *G. pentaphylla* collected from central and eastern populations after spraying with Dragendorff's reagent: (1) Trat, (2) Chanthaburi, (3) Rayong, (4) Chon Buri, (5) Nakhon Nayok, (6) Saraburi, (7) Nakhon Ratchasima, (8) Bangkok

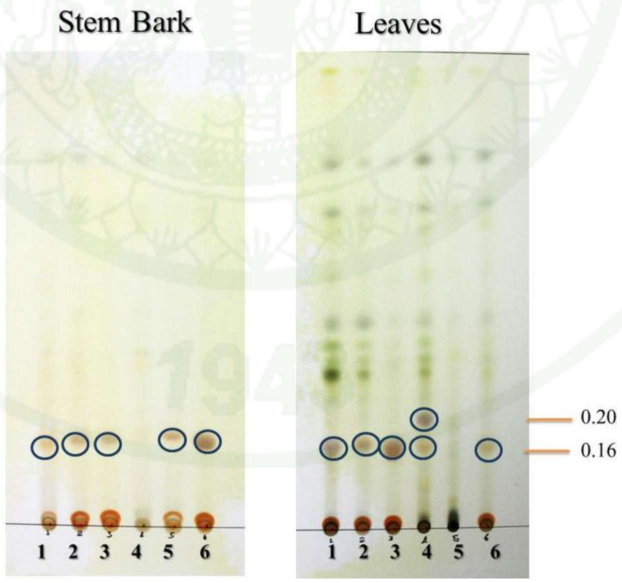



**Figure 27** TLC patterns of stem bark and leaves extracts of *G. pentaphylla* collected from southern populations after spraying with Dragendorff's reagent: (1) Pangnga, (2) Trang, (3) Krabi, (4) Nakhon Si Thammarat, (5) Satun, (6) Songkhla

Variability of results of secondary metabolites testing of plants could be induced by a number of factors such as age, climate, habitat, plant part, season, chemical race of plants, etc. (Harborne, 1988 ). However, this was similar to the finding of Vajrodaya (1998), who isolated alkaloids containing in Thai *Glycosmis* especially carbazole alkaloids and indole alkaloids.

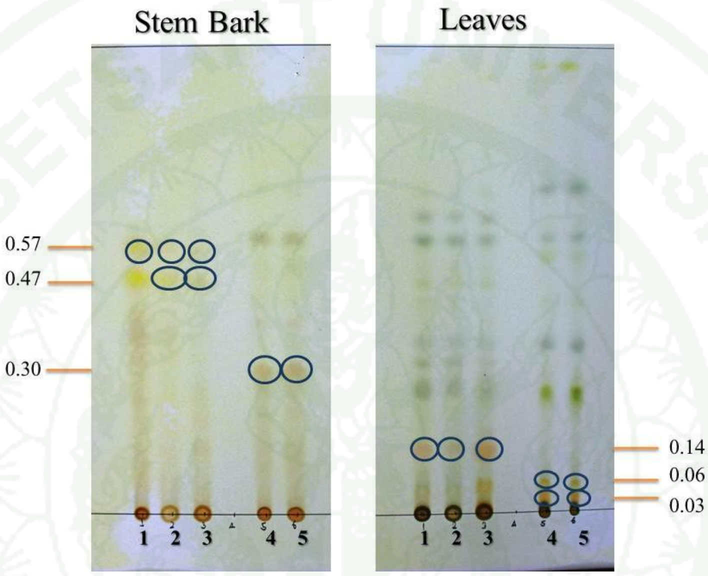



**Figure 28** TLC patterns of stem bark and leaves extracts of *G. parva* and *G. mauritiana* collected from different populations after spraying with Dragendorff's reagent

*G. parva*:

(1) Nakhon Ratchasima (Haew Narok Waterfall)

(2) Nakhon Ratchasima (Sakaerat)

(3) Rayong

*G. mauritiana*:

(4) Prachuap Khiri Khan (Praya Nakhon Cave)

(5) Prachuap Khiri Khan (Laem Sala)

2.1.2 Screening for steroids and terpenoid

Steroids and terpenoids test were done by spraying TLC plate with Anisaldehyde-sulfuric acid reagent. After heating TLC plate at the temperature 110 °C until maximal visualization of the spots. The result showed that stem bark and leaves of all sample gave colored spots from violet, blue and grey green, indicated the all population of *Glycosmis* contain steroids and terpenoid (Table 10, 11 and Figure 29, 30 and 31).

Stem bark extracts of *G. pentaphylla* collecting from central and eastern of Thailand gave similar patterns which could be conbined into 4 groups i.e. group I the collections from populations 1 (Trat), 2 (Chanthaburi) and 4 (Chonburi) group II the collections from populations 5 (Nakhon Nayok), 6 (Saraburi) and 7 (Nakhonrathasima : Haew Narok), group 3 the individual sample from population 3 (Rayong) and group IV the individual sample from population 8 (Bangkok).
All of stem bark extracts of *G. pentaphylla* collecting from the south of Thailand gave different patterns.

Leaves extracts of *G. pentaphylla* collecting from central and eastern of Thailand gave similar patterns only two collections from 2 (Chanthaburi) and 3 (Rayong). Leaves extracts of *G. pentaphylla* collected from the south of Thailand gave similar patterns only two collections from 1 (Pangnga) and 2 (Trang).

Steroids and terpenoids test for the stem bark and leaves extracts of *G. parva* showed different patterns in all collections. Contrast to stem bark and leaves extracts of all *G. mauritiana* collections which showed similarity of steroids and terpenoids patterns in the same organ (stem bark and leaves) collected from different populations in the south.

These results are accordant to report from Sreejith *et al*. (2012), who documented that there are triterpenoids in *G. pentaphylla*, and Chakravarty *et al.* (1996) could isolated triterpenoid from *G. arborea* (later synonym of *G. pentaphylla*).

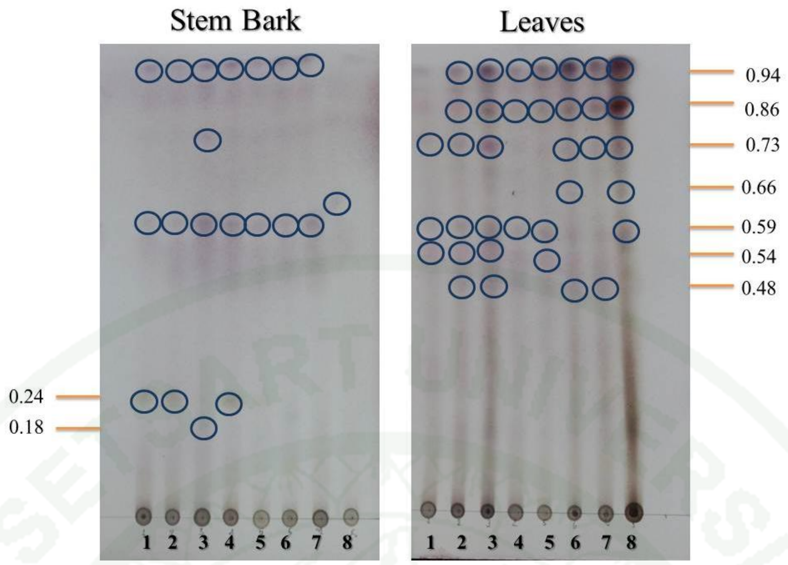



**Figure 29** TLC patterns of stem bark and leaves extracts of *G. pentaphylla* collected from central and eastern populations after spraying with Anisaldehyde-sulfuric acid: (1)Trat, (2) Chanthaburi, (3) Rayong, (4) Chon Buri, (5) Nakhon Nayok, (6) Saraburi, (7) Nakhon Ratchasima, (8) Bangkok

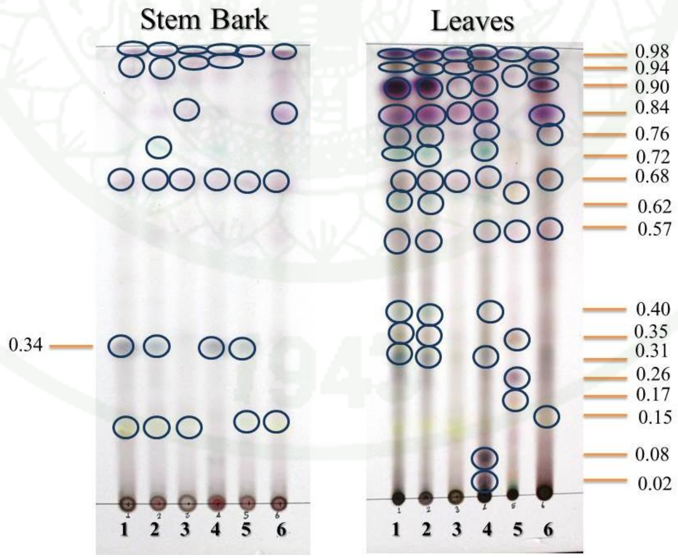



**Figure 30** TLC patterns of stem bark and leaves extracts of *G. pentaphylla* collected from southern populations after spraying with Anisaldehyde-sulfuric acid: (1) Phangnga, (2) Trang, (3) Krabi, (4) Nakhon Si Thammarat, (5) Satun, (6) Songkhla

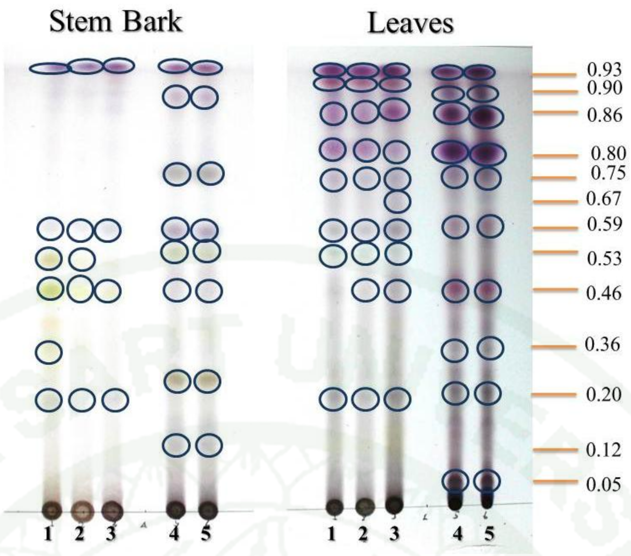



**Figure 31** TLC patterns of stem bark and leaves extracts of *G. parva* and *G. mauritiana* collected from different populations after spraying with Anisaldehyde-sulfuric acid

*G. parva*:

(1) Nakhon Ratchasima (Haew Narok Waterfall)

(2) Nakhon Ratchasima (Sakaerat)

(3) Rayong

*G. mauritiana*:

(4) Prachuap Khiri Khan (Praya Nakhon Cave)

(5) Prachuap Khiri Khan (Laem Sala)

2.1.3 Screening for coumarin

For detection of coumarin, TLC plate was sprayed with 10% NaOH and exposed with UV light at long wavelength (365 nm). There was no presence of yellow-green fluorescence on TLC plates. The result indicated that stem bark and leaves of all populations did not contain with coumarin (Table 10 and 11).

The results agree with modernized treatment of Auratioideae subfamily was given from phylogenetic based on taxonomic structure and chemical evidence. *Glycosmis* was placed in the *Bergera* Alliance, the genera in this alliance are all unarmed and bear pinnate leaves with alternate leaflet, unwinged petiole and rachis, carbazole alkaloids present but only coumarins are not present in the Genus *Glycosmis* (Samuel *et al*., 2001; Mou and Zhang, 2009).

**Table 10** Phytochemical screening of lipophilic stem bark extracts from different populations of *G. pentaphylla*, *G. parva* and *G. mauritiana*

| Species /population | Group of Secondary metabolites | | | Voucher /locality |
|---|---|---|---|---|
| | Alkaloids | Terpenoids | Coumarins | |
| *G. pentaphylla* population 1 | - | + | - | W. Aiyakool 48 Trat |
| *G. pentaphylla* population 2 | - | + | - | W. Aiyakool 42 Chanthaburi |
| *G. pentaphylla* population 3 | - | + | - | W. Aiyakool 46 Rayong |
| G. pentaphylla population 4 | - | + | - | W. Aiyakool 33 Chon Buri |
| *G. pentaphylla* population 5 | - | + | - | W. Aiyakool 40 Nakhon Nayok |
| *G. pentaphylla* population 6 | + | + | - | W. Aiyakool 43 Saraburi |
| *G. pentaphylla* population 7 | + | + | - | W. Aiyakool 44 Nakhon Ratchasima |
| *G. pentaphylla* population 8 | - | + | - | W. Aiyakool 49 Bangkok |
| *G. pentaphylla* population 9 | + | + | - | W. Aiyakool 35 Phangnga |
| *G. pentaphylla* population 10 | + | + | - | W. Aiyakool 36 Trang |
| *G. pentaphylla* population 11 | + | + | - | W. Aiyakool 39 Krabi |
| *G. pentaphylla* population 12 | - | + | - | W. Aiyakool 38 Nakhon Si Thammarat |

**Table 10** (Continued)

| Species /population | Group of Secondary metabolites | | | Voucher /locality |
|---|---|---|---|---|
| | Alkaloids | Terpenoids | Coumarins | |
| *G. pentaphylla* population 13 | + | + | - | W. Aiyakool 37 Satun |
| *G. pentaphylla* population 14 | + | + | - | W. Aiyakool 34 Songkhla |
| *G. parva* population 1 | + | + | - | W. Aiyakool 45 Haeo Narok Waterfall, |
| *G. parva* population 2 | + | + | - | W. Aiyakool 47 Rayong |
| *G. parva* population 3 | + | + | - | W. Aiyakool 41 Sakaerat |
| *G. mauritiana* population 1 | + | +++ | - | W. Aiyakool 50 Prachuap Khiri Khan |
| *G. mauritiana* population 2 | + | +++ | - | W. Aiyakool 51 Prachuap Khiri Khan |

**Remarks**: - negative test, + positive test (low level), +++ positive test (high level)

**Table 11** Phytochemical screening of lipophilic leaves extracts from different populations of *G. pentaphylla*, *G. parva* and *G. mauritiana*

| Species /population | Group of Secondary metabolites | | | Voucher /locality |
|---|---|---|---|---|
| | Alkaloids | Terpenoids | Coumarins | |
| *G. pentaphylla* population 1 | - | + | - | W. Aiyakool 48 Trat |
| *G. pentaphylla* population 2 | - | +++ | - | W. Aiyakool 42 Chanthaburi |
| *G. pentaphylla* population 3 | - | +++ | - | W. Aiyakool 46 Rayong |
| G. pentaphylla population 4 | - | + | - | W. Aiyakool 33 Chon Buri |
| *G. pentaphylla* population 5 | - | + | - | W. Aiyakool 40 Nakhon Nayok |
| *G. pentaphylla* population 6 | + | + | - | W. Aiyakool 43 Saraburi |
| *G. pentaphylla* population 7 | + | + | - | W. Aiyakool 44 Nakhon Ratchasima |
| *G. pentaphylla* population 8 | - | + | - | W. Aiyakool 49 Bangkok |
| *G. pentaphylla* population 9 | + | +++ | - | W. Aiyakool 35 Phangnga |
| *G. pentaphylla* population 10 | + | +++ | - | W. Aiyakool 36 Trang |
| *G. pentaphylla* population 11 | + | +++ | - | W. Aiyakool 39 Krabi |
| *G. pentaphylla* population 12 | + | +++ | - | W. Aiyakool 38 Nakhon Si Thammarat |

**Table 11** (Continued)

| Species /population | Group of Secondary metabolites | | | Voucher /locality |
|---|---|---|---|---|
| | Alkaloids | Terpenoids | Coumarins | |
| *G. pentaphylla* population 13 | - | + | - | W. Aiyakool 37 Satun |
| *G. pentaphylla* population 14 | + | + | - | W. Aiyakool 34 Songkhla |
| *G. parva* population 1 | + | +++ | - | W. Aiyakool 45 Haeo Narok Waterfall, |
| *G. parva* population 2 | + | +++ | - | W. Aiyakool 47 Rayong |
| *G. parva* population 3 | + | +++ | - | W. Aiyakool 41 Sakaerat |
| *G. mauritiana* population 1 | + | +++ | - | W. Aiyakool 50 Prachuap Khiri Khan |
| *G. mauritiana* population 2 | + | +++ | - | W. Aiyakool 51 Prachuap Khiri Khan |

**Remarks**: - negative test, + positive test (low level), +++ positive test (high level)

2.1.4 Chemical profiles detected by UV light

All stem bark and leaves extracts were analyzed on the basis of their TLC patterns. The chemical constituents were detected by visualization under UV light both at short wavelength (254 nm) and long wavelength (365 nm).

**TLC patterns detected under UV 254 nm**

Base on TLC patterns which were detected under UV 254 nm, All the dark spots were observed. It was shown that *G. pentaphylla* collected from central and eastern of Thailand showed dark spot at the same $R_f$ value 0.10 in all stem bark and leaves extracts of the populations 1-7 (Trat, Chanthaburi, Rayong, Chon Buri, Nakhon Nayok, Saraburi, and Nakhon Ratchasima respectively) except the population 8 (Bangkok). On the other hand, dark spots at $R_f$ value 0.38 could be observed in population 6 (Saraburi) and 7 (Nakhon Ratchasima). TLC patterns of population 6 and 7 are similar in each organ (stem bark and leaves). So, from stem bark extracts of all samples collected from central and eastern of Thailand, it could be combined into 4 groups i.e. group I collections from populations 1, 2, 4 and 5, group II collections from populations 6 and 7, group III and IV collections from populations 3 and 8 respectively. From stem bark extracts from southern collections, only the populations 3 (Krabi) and 6 (Songkla) showed similar patterns. The other populations showed different patterns (Figure 32, 33).

Leaves extracts of *G. pentaphylla* collected from central and eastern of Thailand showed dark spots and could be combined to be 5 groups, i. e. group I the collections from populations 2 and 4, group II populations 3 and 5, group III populations 6 and 7 and different individuals or group IV and group 5 are populations 1, 8 respectively. Leaves extracts from southern collections, all collections showed different patterns (Figure 32, 33).

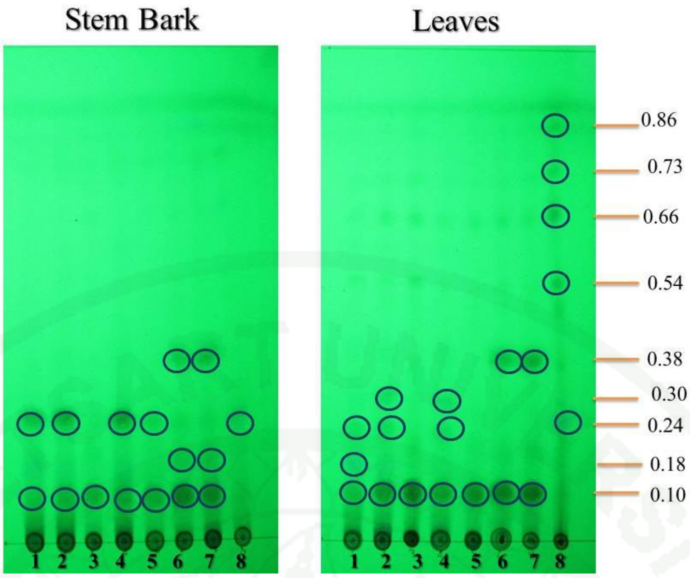



**Figure 32** TLC patterns under UV 254 nm of stem bark and leaves of *G. pentaphylla* collected from central and eastern populations: (1)Trat, (2) Chanthaburi, (3) Rayong, (4) Chon Buri, (5) Nakhon Nayok, (6) Saraburi, (7) Nakhon Ratchasima, (8) Bangkok

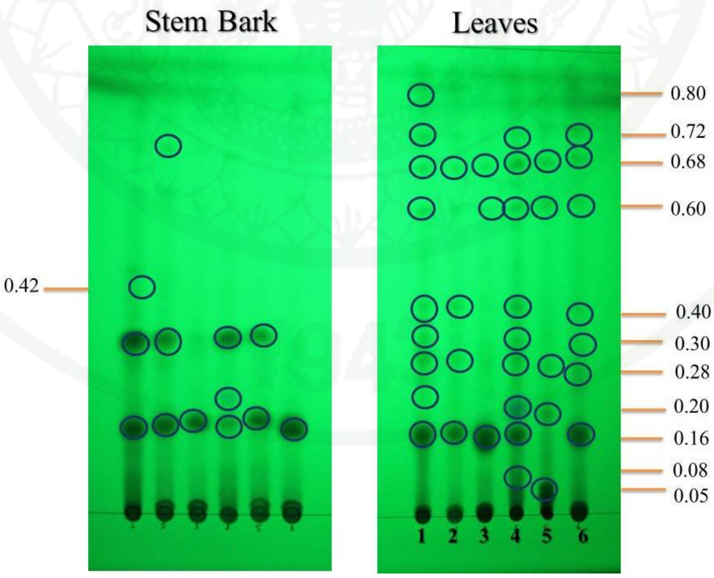



**Figure 33** TLC patterns under UV 254 nm of stem bark and leaves of *G. pentaphylla* collected from southern populations: (1) Pangnga, (2) Trang, (3) Krabi, (4) Nakhon Si Thammarat, (5) Satun, (6) Songkhla population.

Dark spots from stem bark extracts of *G. parva* in all collections gave different patterns contrast to leaves extracts which showed similar patterns in all collections. Interestingly, all collections of *G. mauritiana* gave similar patterns of dark spots in the same organs (stem bark and leaves) (Figure 34).

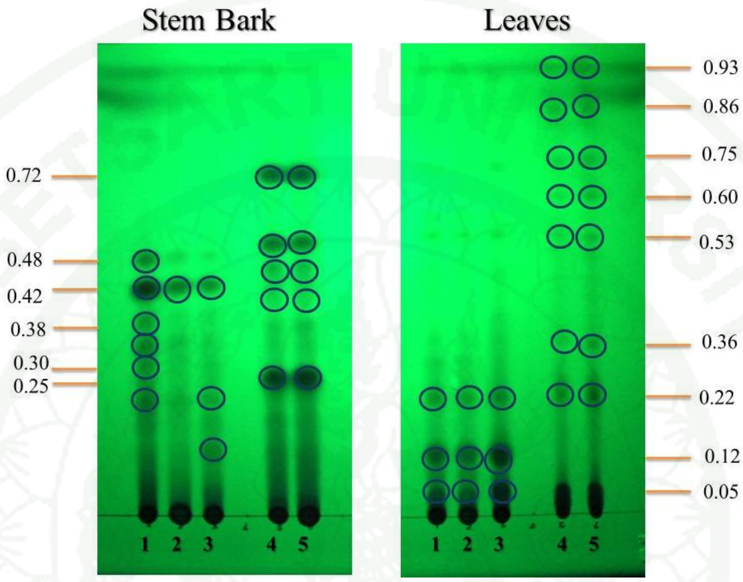



**Figure 34** TLC patterns under UV 254 nm of stem bark and leaves of *G. parva* and *G. mauritiana* collected from different populations

*G. parva*:

(1) Nakhon Ratchasima (Haew Narok Waterfall)

(2) Nakhon Ratchasima (Sakaerat)

(3) Rayong

*G. mauritiana*:

(4) Prachuap Khiri Khan (Praya Nakhon Cave)

(5) Prachuap Khiri Khan (Laem Sala)

### TLC patterns detected under UV 365 nm

TLC patterns were detected under UV 365 nm, all colorful fluorescent spots were observed except red fluorescences of chlorophylls.

*G. pentaphylla* collected from central and eastern of Thailand showed blue-green fluorescent spots at the same $R_f$ value 0.73 as well as blue-violet fluorescent spots at $R_f$ value 0.38 in stem bark and leaves extracts of all populations 1-8 (Trat, Chanthaburi, Rayong, Chon Buri, Nakhon Nayok, Saraburi, Nakhon Ratchasima and Bangkok respectively). So, these might be used as chemical characters of *G. pentaphylla*. Stem bark extracts from population 2 and 4 showed the same patterns of fluorescent spots i.e. blue-violet spots at the $R_f$ value 0.18, 0.24, 0.38 and blue spots at $R_f$ value 0.84 (Figure 35). There are similar patterns of fluorescent spots in populations 6 and 7, blue-violet fluorescent spots at $R_f$ value 0.10, 0.24, 0.38, blue-green at $R_f$ value 0.73 and blue fluorescent spots at $R_f$ value 0.78. In contrast to the collections from the south which blue-green fluorescent spots could be detected only in stem bark extracts at $R_f$ value 0.76, but in leaf extracts these blue-green fluorescent spots could be detected only in the collection from the populations 3-6 (Krabi, Nakhon Si Thammarat, Satun and Songkhla respectively) (Figure 36).

In *G. parva*, blue-green spots occurred at $R_f$ value 0.80 in stem bark of populations 1 (Nakhon Ratchasima (Haew Narok)) and 2 (Nakhon Ratchasima(Sakaerat)). In leaves extracts of southern populations, same patterns of fluorescent spots could be observed as violet color at $R_f$ value 0.40 and blue-green at $R_f$ value 0.80. From stem bark extract of *G. mauritiana* there were violet spots at $R_f$. Value 0.12 and blue-green spots at $R_f$ value 0.80 (Figure 37).

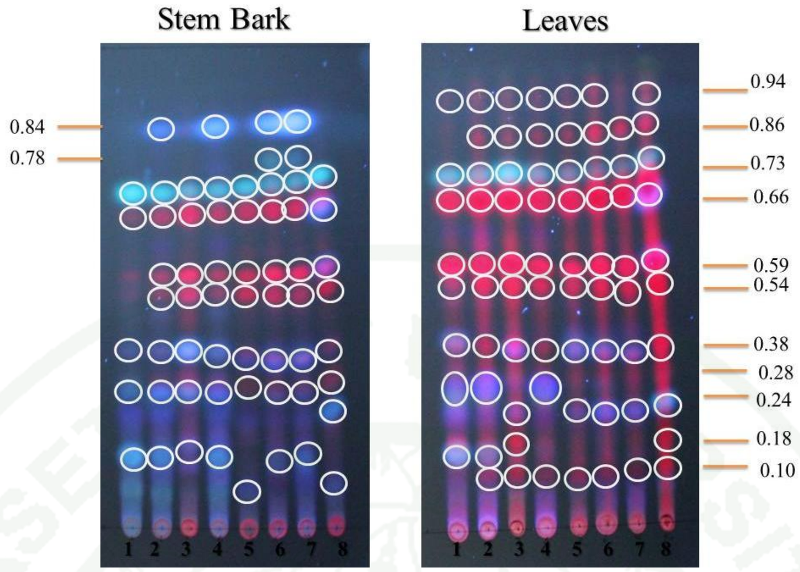



**Figure 35** TLC patterns under UV 365 nm of stem bark and leaves of *G. pentaphylla* collected from central and eastern populations: (1)Trat, (2) Chanthaburi, (3) Rayong, (4) Chon Buri, (5) Nakhon Nayok, (6) Saraburi, (7) Nakhon Ratchasima, (8) Bangkok

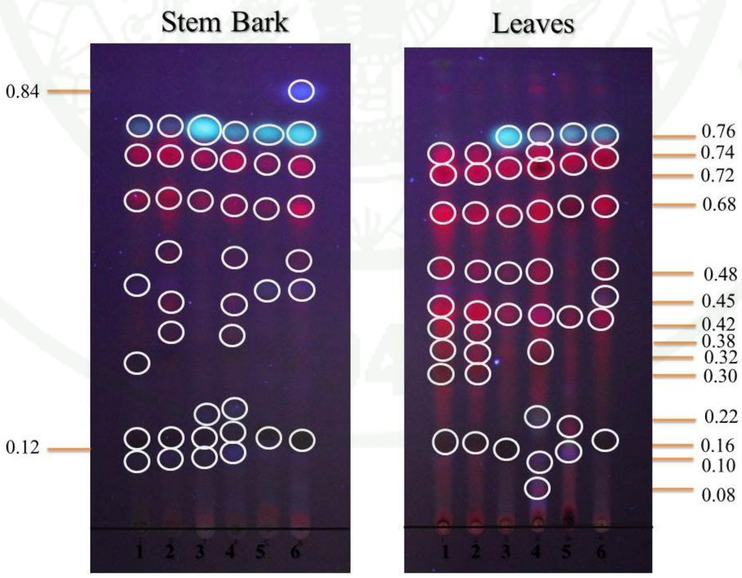



**Figure 36** TLC patterns under UV 365 nm of stem bark and leaves of *G. pentaphylla* collected from southern populations: (1) Pangnga, (2) Trang, (3) Krabi, (4) Nakhon Si Thammarat, (5) Satun, (6) Songkhla

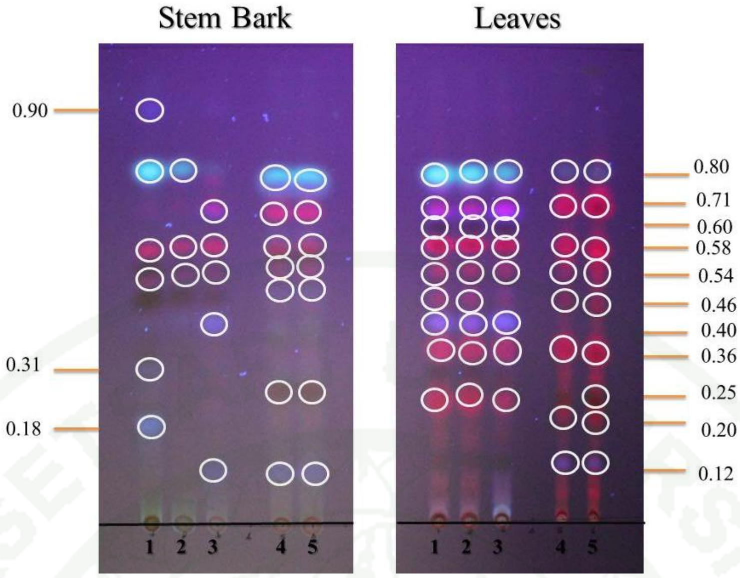



**Figure 37** TLC patterns under UV 365 nm of stem bark and leaves of *G. parva* and *G. mauritiana* collected from different populations

*G. parva*:

(1) Nakhon Ratchasima (Haew Narok Waterfall)

(2) Nakhon Ratchasima (Sakaerat)

(3) Rayong

*G. mauritiana*:

(4) Prachuap Khiri Khan (Praya Nakhon Cave)

(5) Prachuap Khiri Khan (Laem Sala)

2.2 High performance liquid chromatography (HPLC)

2.2.1 *G. pentaphylla*

*G. pentaphylla* represents the most widespread and variable species within the genus. The HPLC profiles of stem bark and leaves, dominant peaks usually showed before retention time 10 min (Figure 38 - 41). From patterns of dominant peaks both in stem bark and leaves extracts, *G. pentaphylla* collected from central and eastern populations could be combined into the same group. Moreover, the patterns of dominant peaks in stem bark and leaves extracts looked similar. Contrast to southern populations of *G. pentaphylla* which did not show only different patterns of dominant peaks in the same organs (stem bark and leaves) (Figure 42, 43) compared with the central and eastern populations, but there were different patterns of dominant peaks in the different organs (stem bark and leaves) of the southern populations (Figure 43-45).

From patterns of dominant peaks in leaves extracts, *G. pentaphylla* collected from southern populations; can be combined into three groups using similarity of HPLC profile i.e. group I: the population of Phangnga, Trang and Songkkla, group II: the population of Nakhon Si Thammarat and Satun, group III: the population of Krabi (Figure 44 and 45).

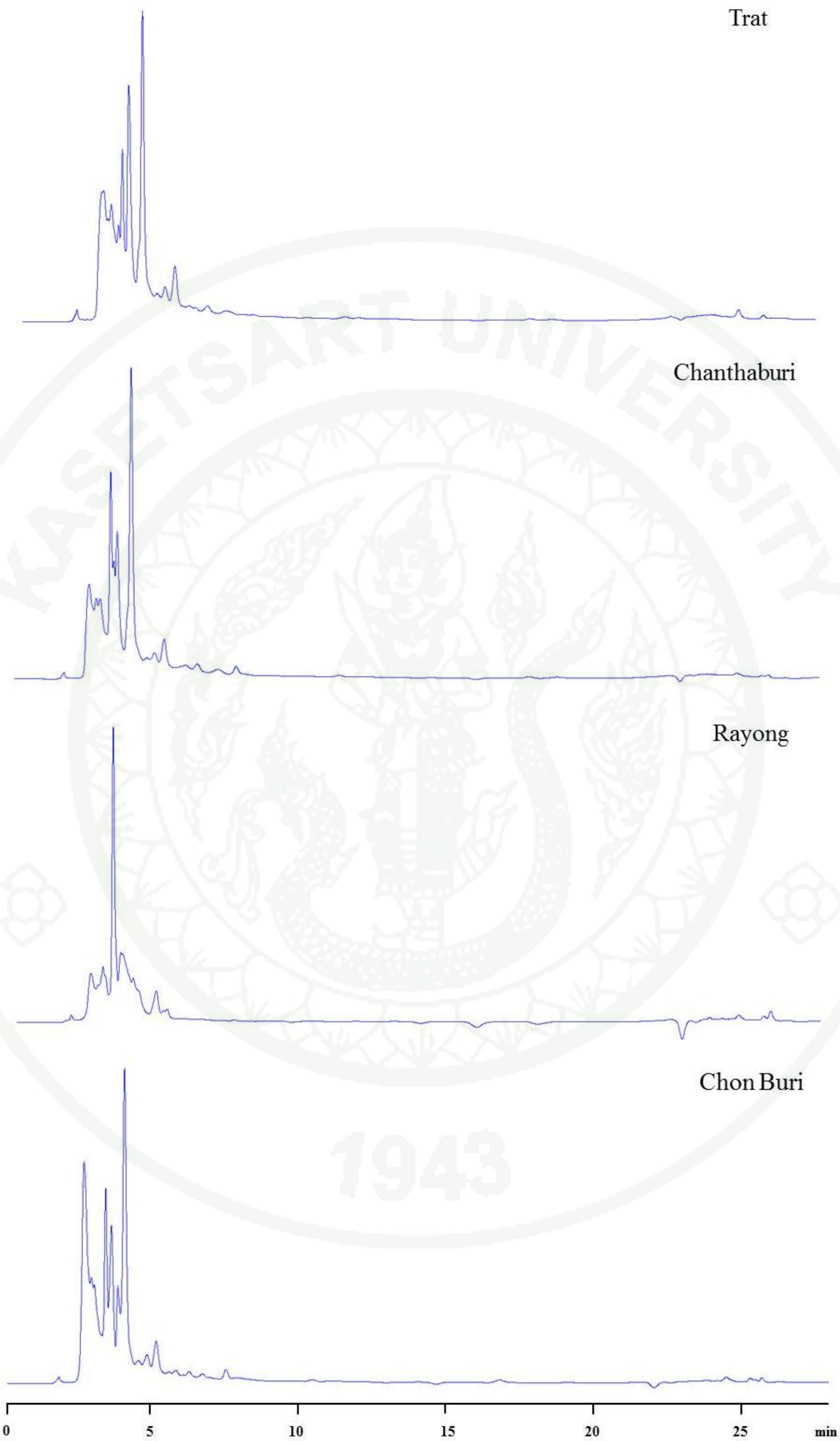



**Figure 38** HPLC comparison of stem bark of *G. pentaphylla* collected from central and eastern populations

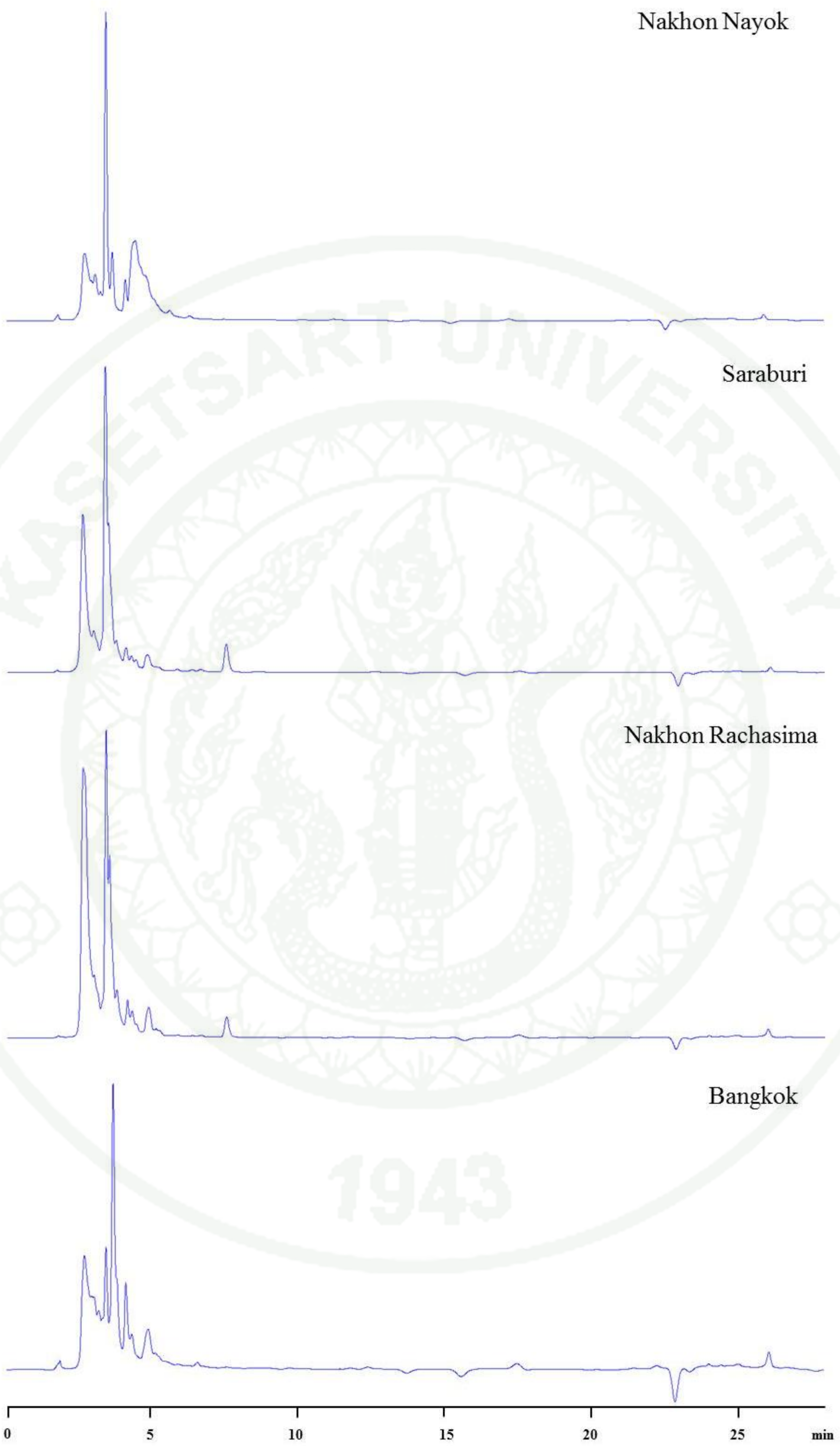



**Figure 39** HPLC comparison of stem bark of *G. pentaphylla* collected from central and eastern populations

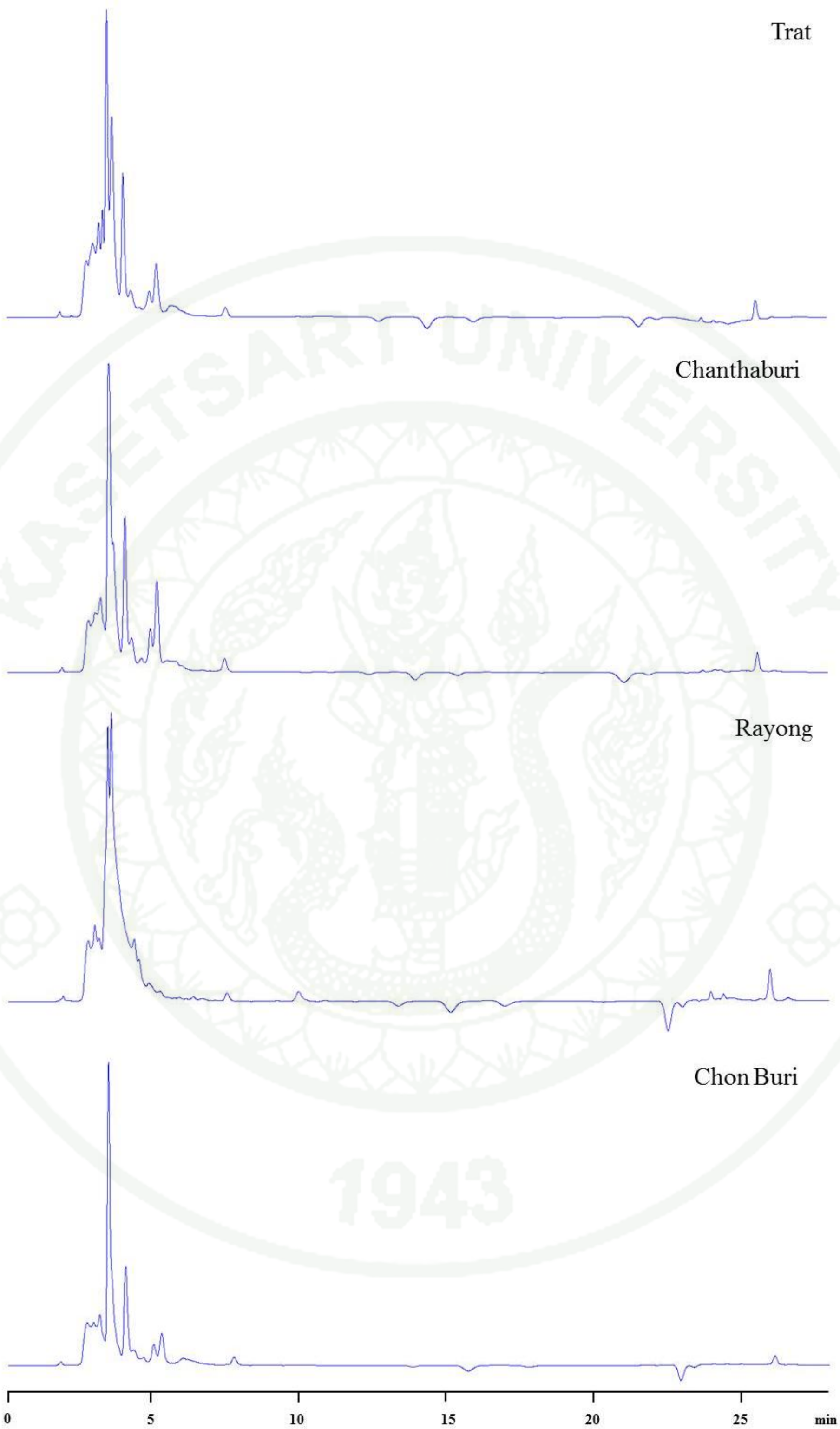



**Figure 40** HPLC comparison of leaves of *G. pentaphylla* collected from central and eastern populations

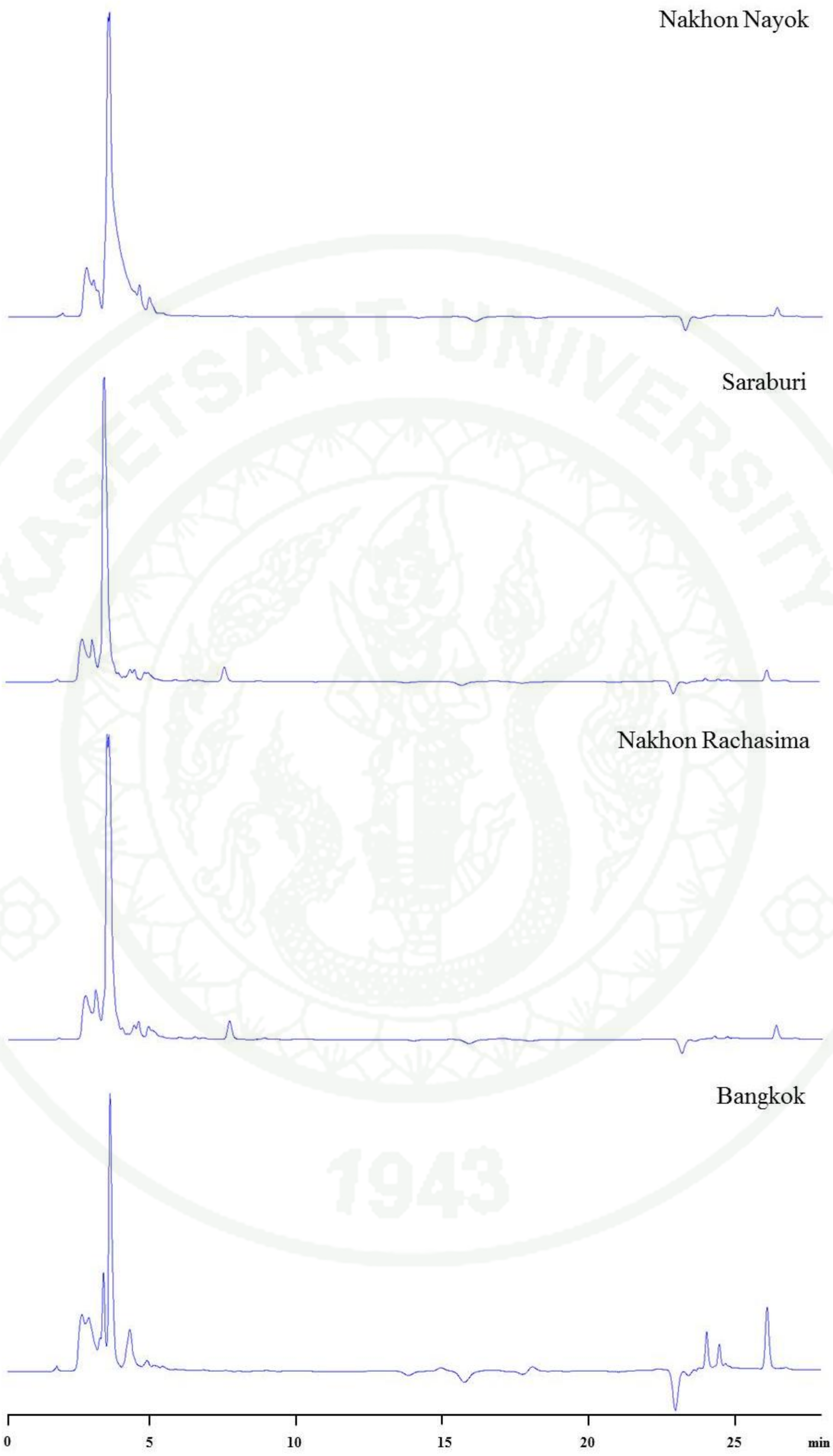



**Figure 41** HPLC comparison of leaves of *G. pentaphylla* collected from central and eastern populations

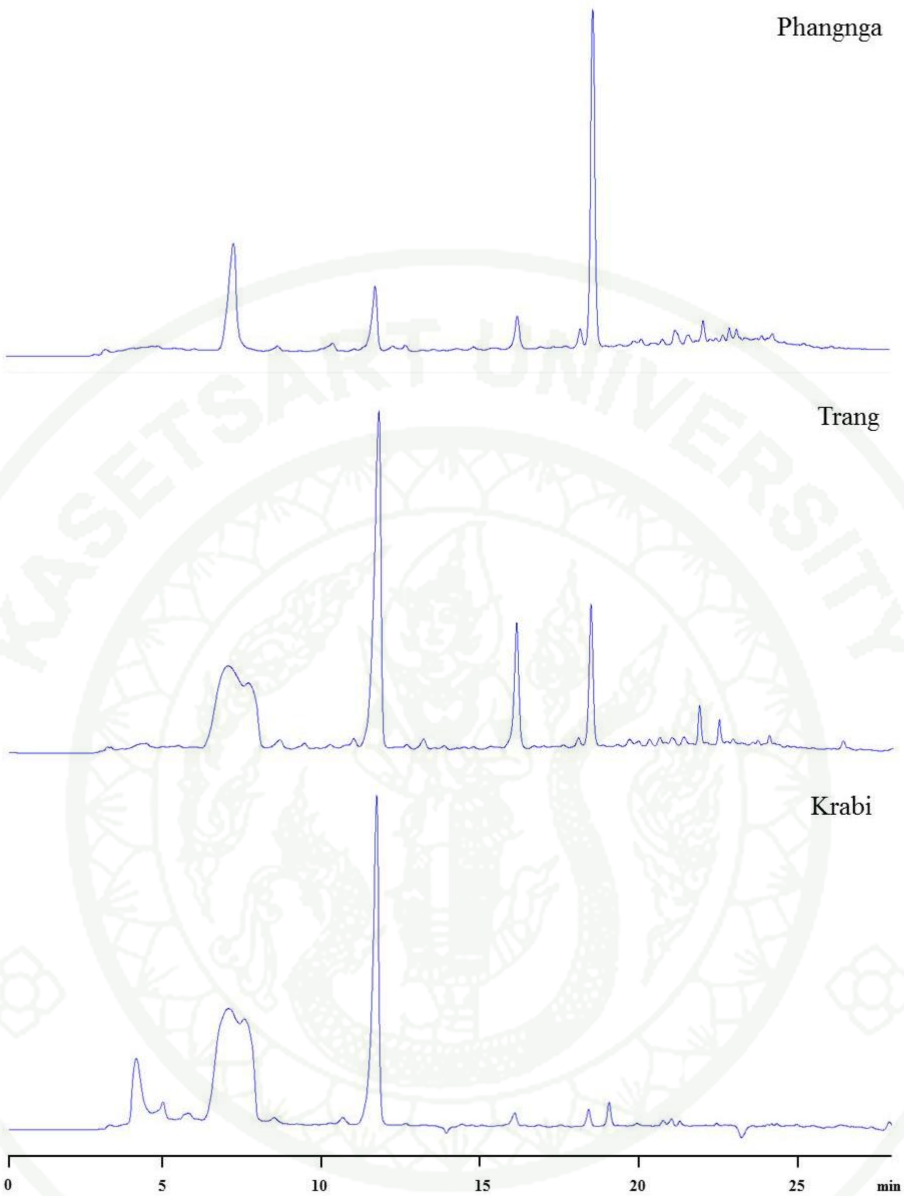



**Figure 42** HPLC comparison of stem bark of *G. pentaphylla* collected from southern populations

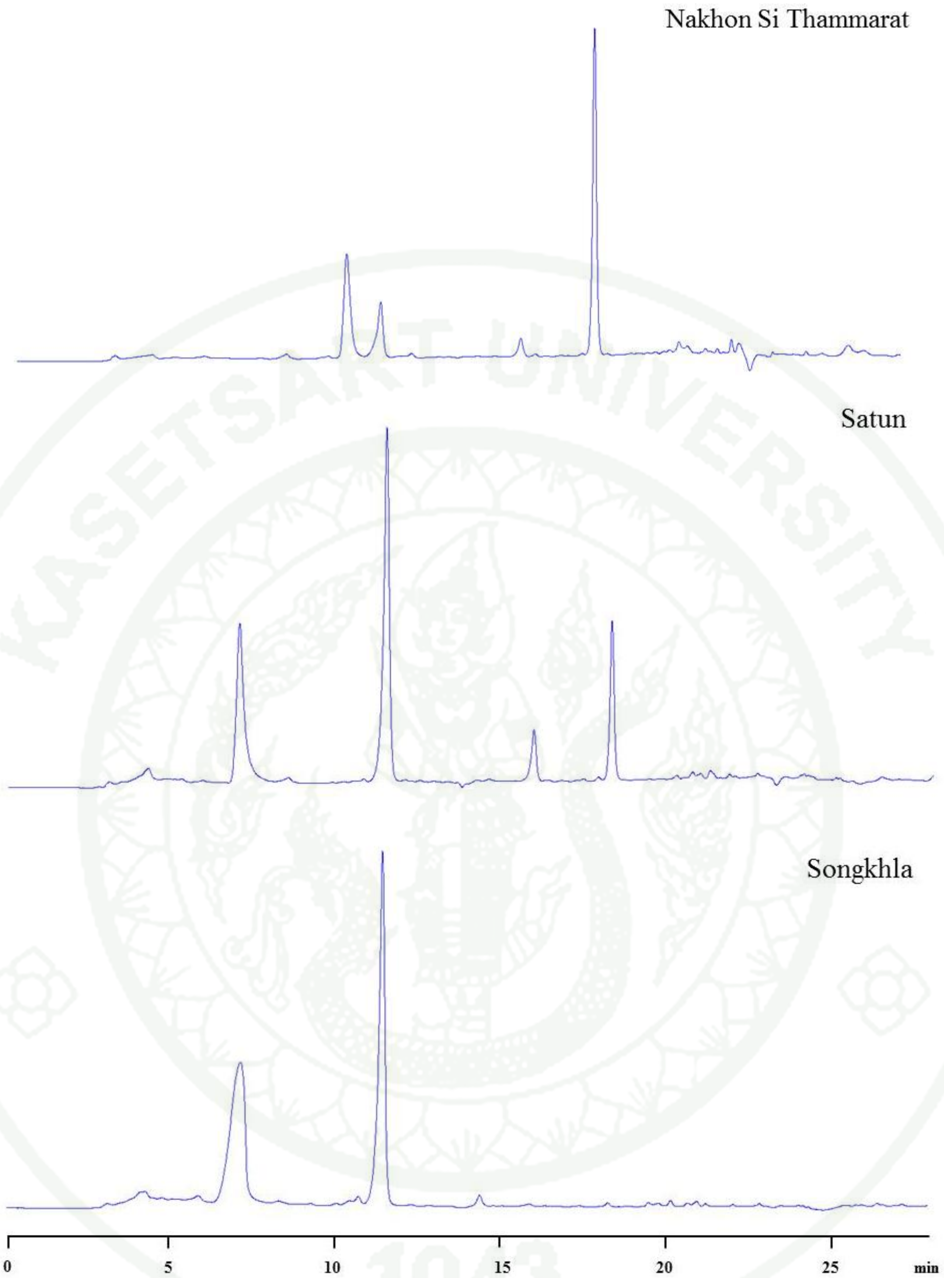



**Figure 43** HPLC comparison of stem bark of *G. pentaphylla* collected from southern populations

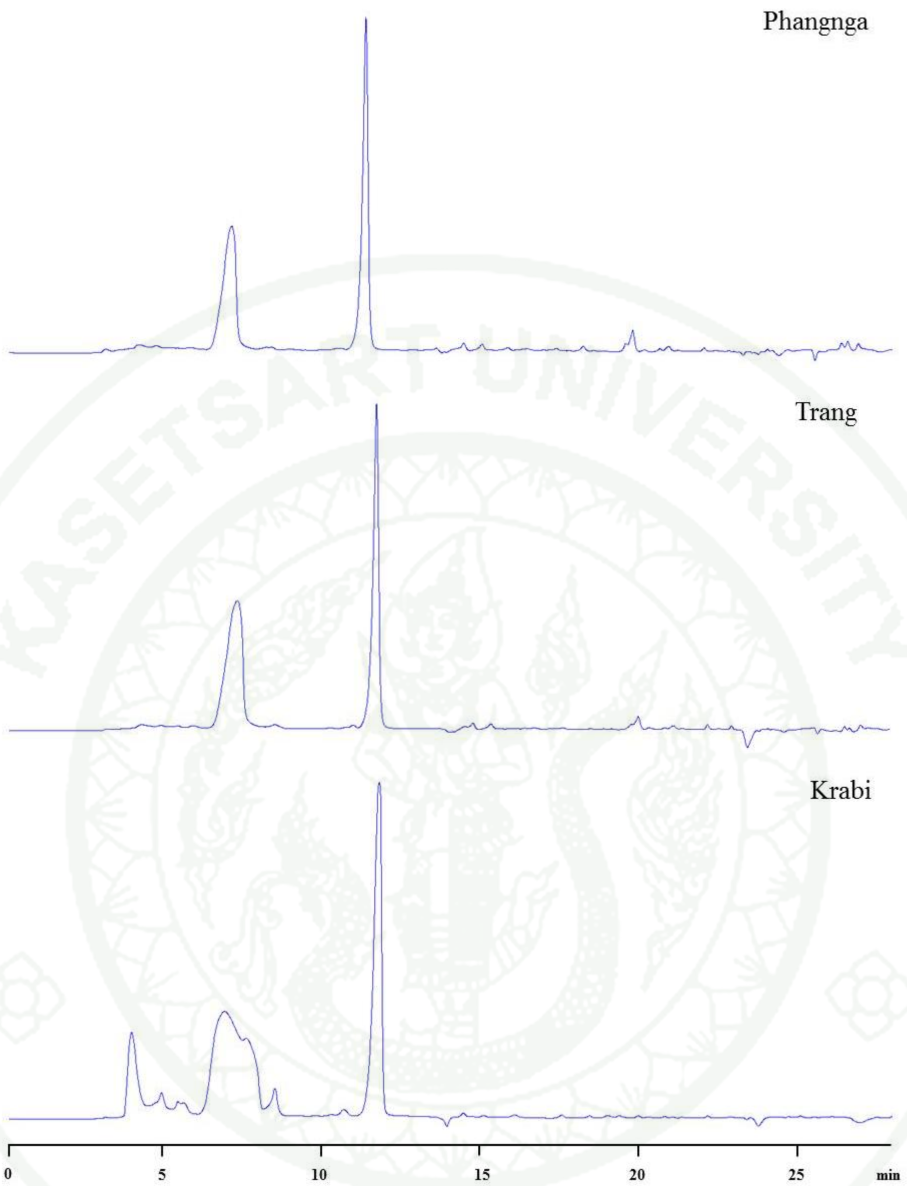



**Figure 44** HPLC comparison of leaves of *G. pentaphylla* collected from southern populations

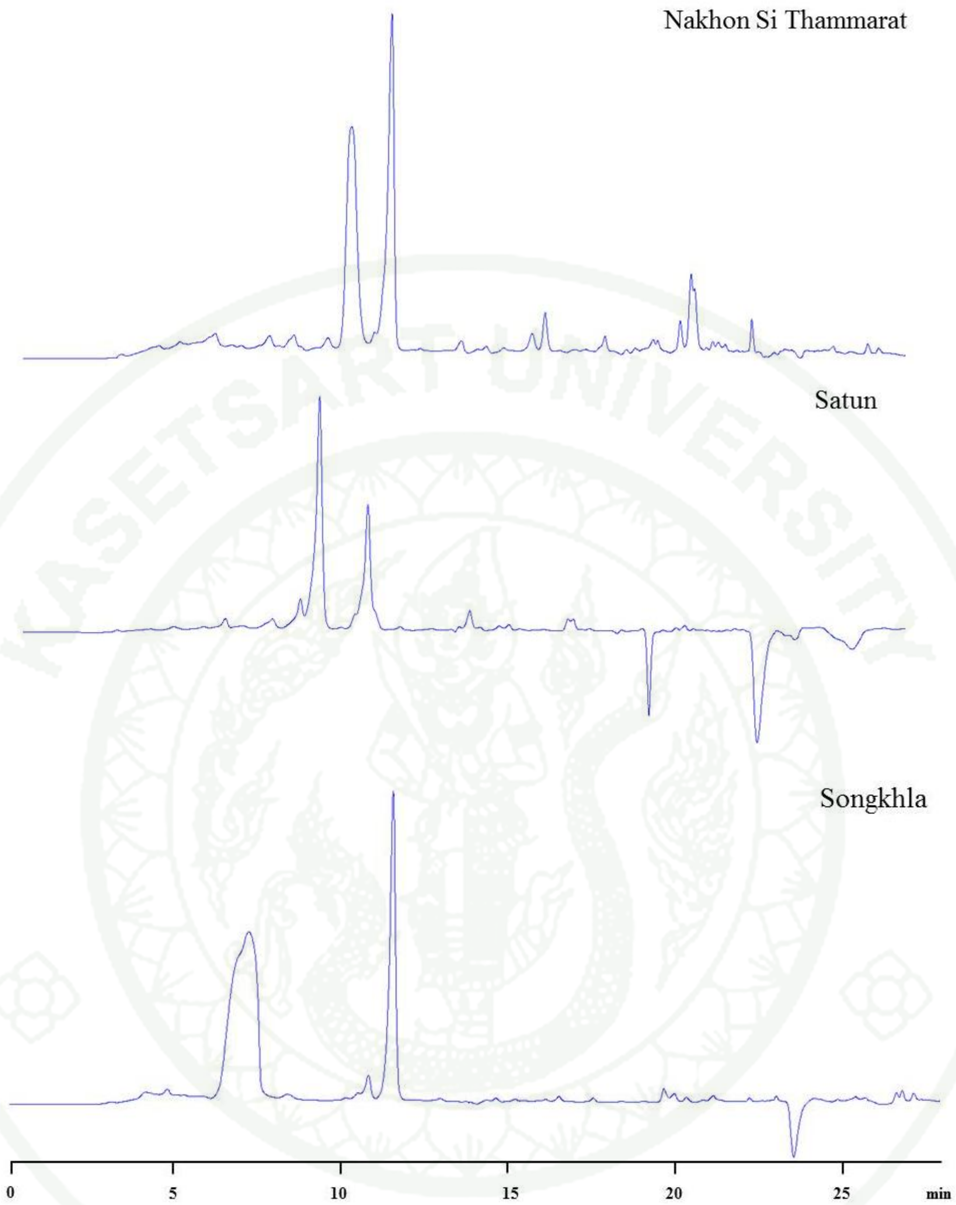



**Figure 45** HPLC comparison of leaves of *G. pentaphylla* collected from southern populations

2.2.2 *G. parva*

From HPLC profiles, The extracts of *G. parva* from different organs (stem bark and leaves) showed different patterns of dominant peaks (Figure 46 and 47). Also, the patterns of dominant peaks in the same organ of *G. parva* collected from different provinces are similar.

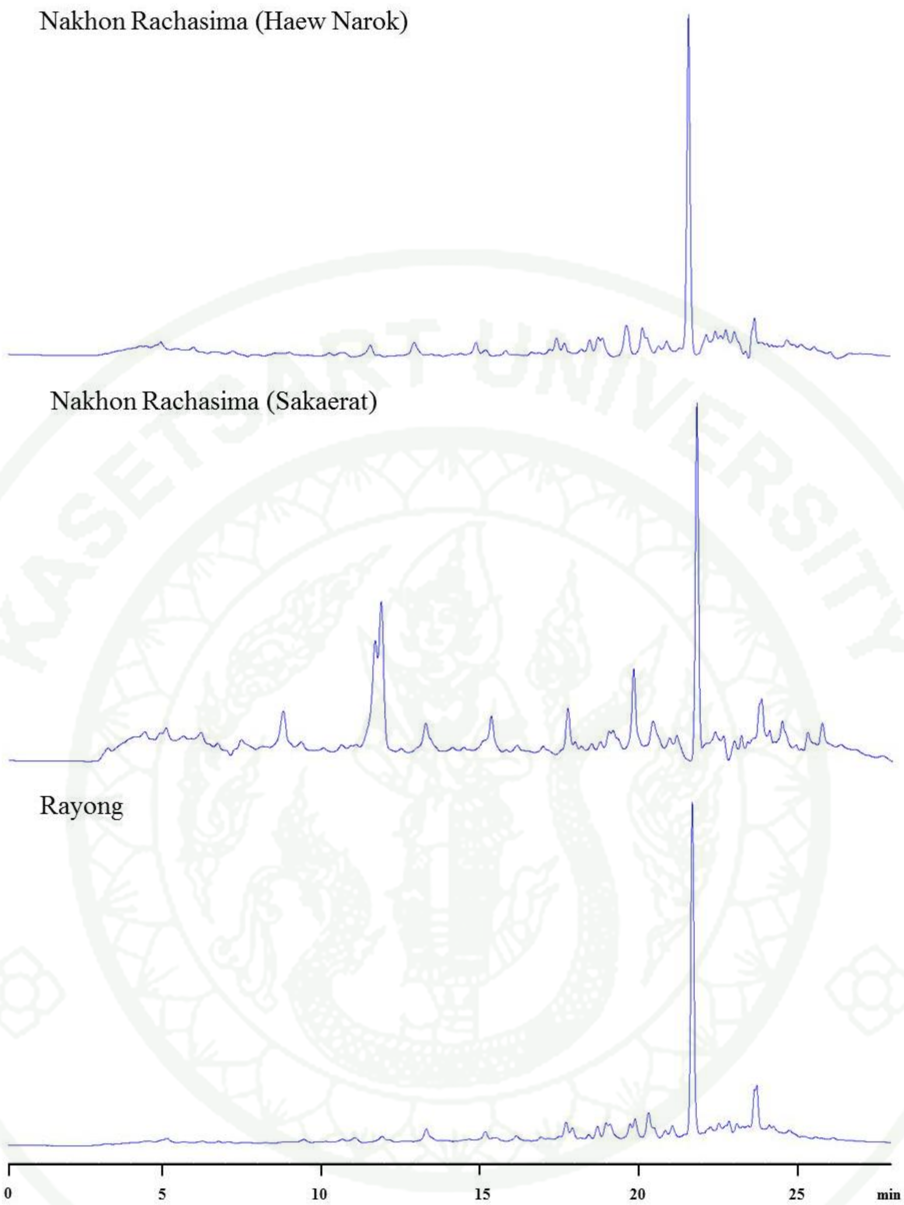



**Figure 46** HPLC comparison of stem bark of *G. parva* collected from different populations

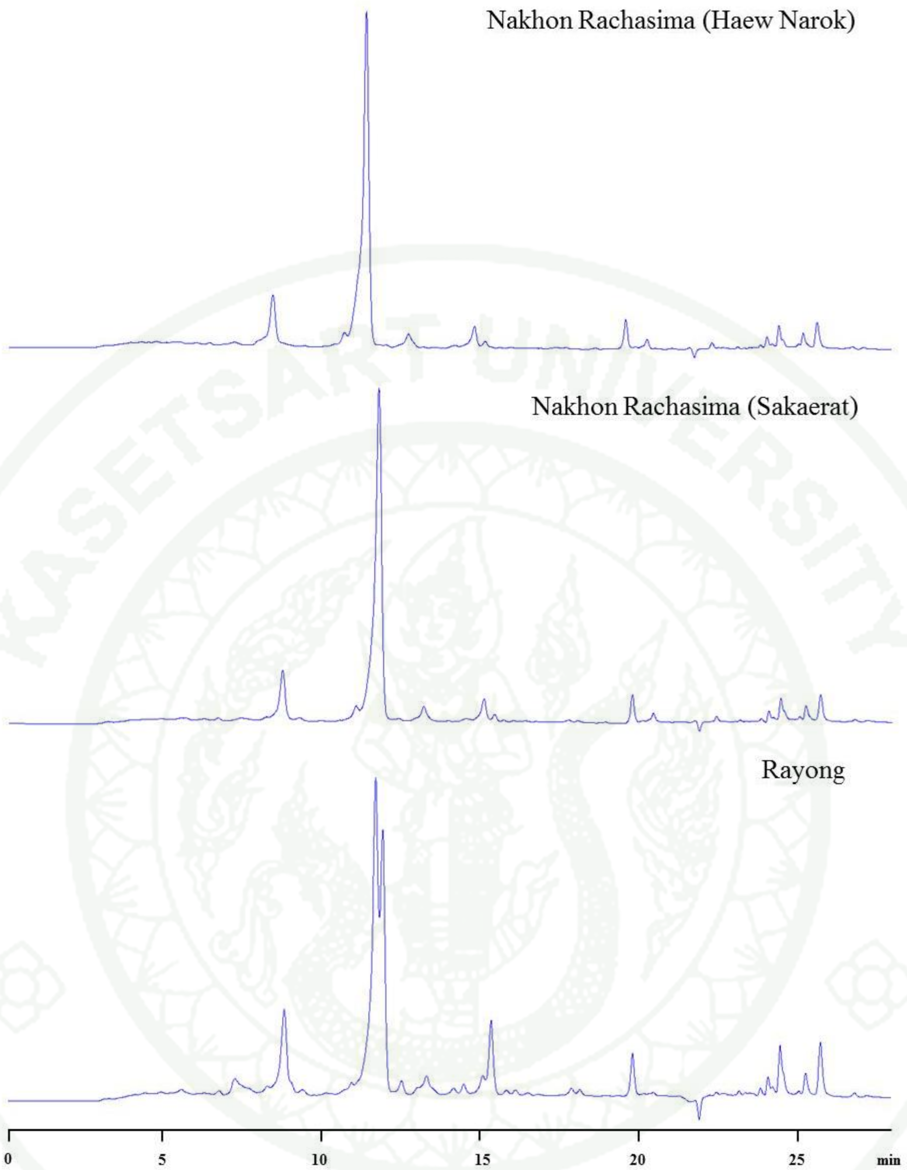



**Figure 47** HPLC comparison of leaves of *G. parva* collected from different populations

2.2.3 *G. mauritiana*

From HPLC profiles, the extracts of *G. mauritiana* from different organs (stem bark and leaves) showed different patterns of dominant peaks (Figure 48 and 49). Also, the patterns of dominant peaks in the same organ of *G. mauritiana* collected from different provinces are similar.

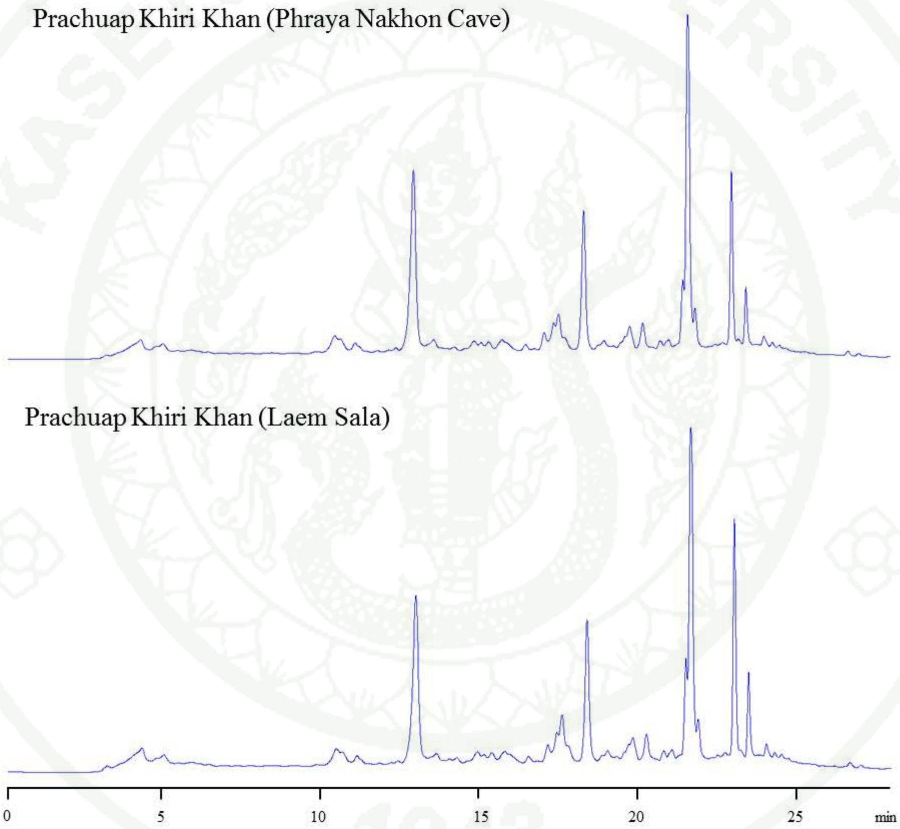



**Figure 48** HPLC comparison of stem bark of *G. mauritiana* collected from different populations

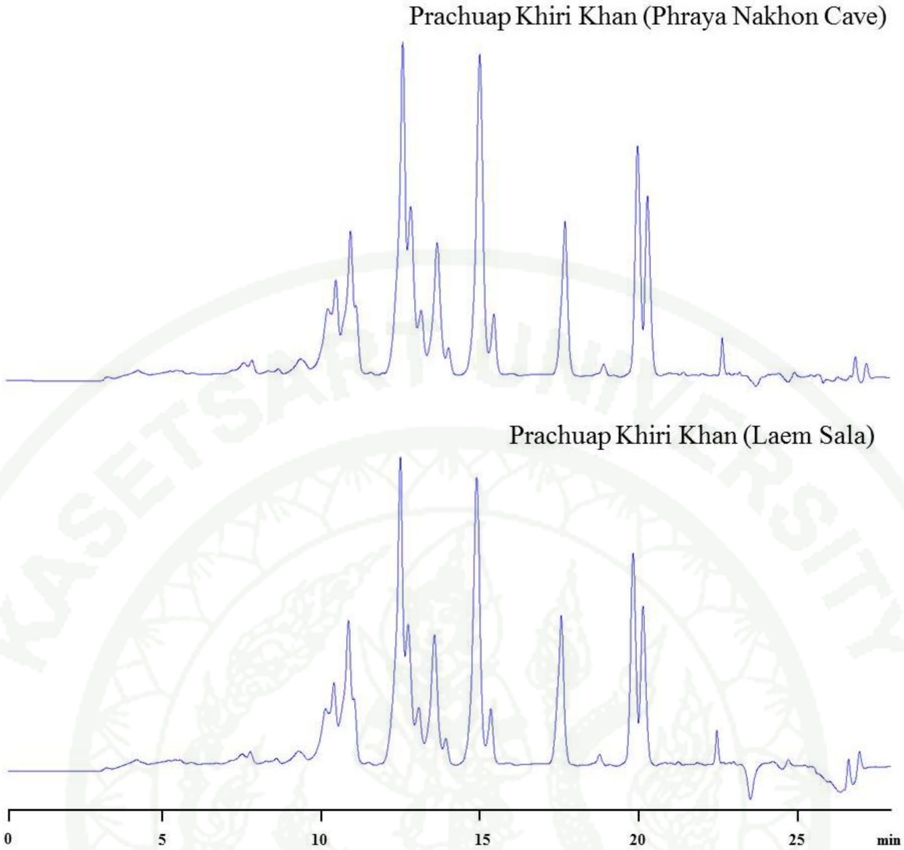



**Figure 49** HPLC comparison of leaves of *G. mauritiana* collected from different populations

# CONCLUSION AND RECOMMENDATION

## Conclusion

1. Morphological study

From result of morphological study within 19 populations of the three groups of *Glycosmis* i.e. *G. pentaphylla*, *G. parva* and *G. mauritiana* and comparing with herbarium specimens throughout the country which deposited in several herbaria in Thailand, these three species can be classified by using ovary and leaves characters.

<u>Key to species of tree groups of *Glycosmis* in Thailand</u>

1. Ovary glabrous
   2. Leaves unifoliolate …………….…………………………………..…...….. *G. parva*
   2. Leaves pinnately compound …………..…..…………….………… *G. pentaphylla*
1. Ovary puberulous ……………………………………………………..….. *G. mauritiana*

*G. pentaphylla* has glabrous ovary and pinnately compound leaves. *G. parva* has glabrous ovary with narrowly elliptic unifoliolate leaf. *G. mauritiana* has puberulent ovary with pinnately compound leaves. The result was supported by Stone (1985, 1994) who classified *Glycosmis* by using leaves, indumentum of androecium and gynoecium, and fruit.

*G. pentaphylla* are mostly variation in number of leaflets; most of them have 5 leaflets, 5-7 leaflet, 3-5 leaflets and 7 leaflets. The blade is narrowly elliptic, elliptic and ovate. The axillary and terminal inflorescences always occur in most individual plant of *G. pentaphylla*, but some populations only axillary or terminal inflorescences can be produced on each individual plant separately.

2. TLC and HPLC analyses

Thin layer chromatography (TLC) showed chemical profiles of alkaloids in stem bark and leaves extracts of all populations of *G. parva*, *G. mauritiana* and also *G. pentaphylla* which collected from Saraburi and Nakhon Ratchasima, Phangnga, Trang, Krabi and Songkla. Anyhow, no alkaloids could be detected from stem bark extracts of *G. pentaphylla* collected from Nakhon Si Thammarat and leaf extract of *G. pentaphylla* collected from Satun. Chemical profiles of steroids and terpenoids in all extracts of the same plant parts of these three *Glycomis* species showed a little differences among populations of *G. parva*, but totally similar in *G. mauritiana*.

Steroids and terpenoid profiles from the same plant parts of *G. pentaphylla* are somehow different among each population which can be grouped into 4 groups. Moreover, the collections from the southern populations are rather similar both in stem bark and leaf extracts only in the populations from Phangnga and Trang.

Coumarin could not be detected in these three *Glycosmis* species from all populations.

Chemical profiles of the same plant parts detected under UV at the wavelength 254 nm showed specific character in leaf extract of *G. pentaphylla* collected from Bangkok. *G. parva* and *G. mauritiana* have specific chemical profiles both in stembark and leaves.

Chemical profiles of the same plant parts detected under UV at the wavelength 365 nm showed specific characters of *G. mauritiana* and also in leaves of *G. parva*, but in *G. pentaphylla* from different populations it was shown that the chemical profiles are not definitely similar.

High performance liquid chromatography (HPLC) showed the difference among three species by dominant peaks at different retention time. The same plant parts (stem barks and leaves) from each population of *G. parva* are similar as well as *G. mauritiana*. HPLC profiles from *G. pentaphylla* are similar in the same plant parts, but somehow vary among populations. Moreover, *G. pentaphylla* from southern populations showed similarity in leaves from the populations of Nakhon Si Thammarat and Satun.

3. Morphological and chemical character

Chemical character can support morphological characters, combine with two characters; present or absent of alkaloids and terpenoids, dominant HPLC peaks, leaves and ovary texture can be used in order to separate three groups of *Glycosmis*.

HPLC profile of leaves of *G. pentaphylla* collected from Krabi showed the difference from the other southern population. This character supported the morphological character Krabi population which has seven leaflets different from the other sample in this species which always have five leaflets.

Chemical characters are stable in *G. mauritiana* both in stem bark and leaves and can be used in order to support morphological characters of *G. mauritiana*.

So, chemical characters are another taxonomic tool which can be used for clarification of taxonomic problem in three groups of *Glycosmis* (*G. pentaphylla, G. parva* and *G. mauritiana*).

**Recommendation**

1. In this study collecting areas were not covered ranges of distribution of the three groups of *Glycosmis*. Thus it was recommended to add wide ranges collecting areas that cover in the future study.

2. The sample of *G. parva* and *G. mauritiana* collected from some populations were founded without corolla and androecium because those organs are strongly caducous. Thus it was recommended to repeat collecting specimens in each population.

3. The chemical character of each organ of each species should be compared with the same organ from herbarium specimens. Unfortunately, Thai herbarium specimens were coated with inorganic chemical in order to prevent decomposition from insects and fungi. Thus, the chemical characters from herbarium specimens might be artifacts.

4. Chemical characters from different chromatographic techniques i.e. TLC and HPLC showed somewhat unrelated. Thus, more samples from each population are needed for comparison.

5. There are many groups of chemical compounds in the stem barks and leaf extracts of *Glycosmis*. Thus, this plant species might have economic value and could be used as a natural source for those compounds. Especially, *G. pentaphylla* are common and distribute widespread more than the other species.

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# APPENDIX

**Preparation of reagents for phytochemical screening**

1. Dragendorff's reagent

Solution A:

| | |
|---|---|
| Bismuth sub nitrate ($BiO(NO_3)\ H_2O$): AR grade Merck | 0.85 g |
| Glacial acetic acid ($CH_3COOH$): AR grade Merck | 10.0 ml |
| Distilled water ($H_2O$): 16 MΩ/cm Millipore | 40.0 ml |

Solution B:

| | |
|---|---|
| Potassium iodide (KI): AR grade Univar | 8.0 g |
| Distilled water ($H_2O$): 16 MΩ/cm Millipore | 20.0 ml |

Solution C:

| | |
|---|---|
| Glacial acetic acid ($CH_3COOH$): AR grade Merck | 20.0 ml |
| Distilled water ($H_2O$): 16 MΩ/cm Millipore | 80.0 ml |

Before use, 5 ml of solution A was mixed with 5 ml of solution B and 100 ml of solution C. Amines and basic heterocycles like pyridine produce brown-orange spots at retention time. Phosphines and crown ethers are also detected (Farnsworth, 1966).

2. Anisaldehyde-sulfuric acid

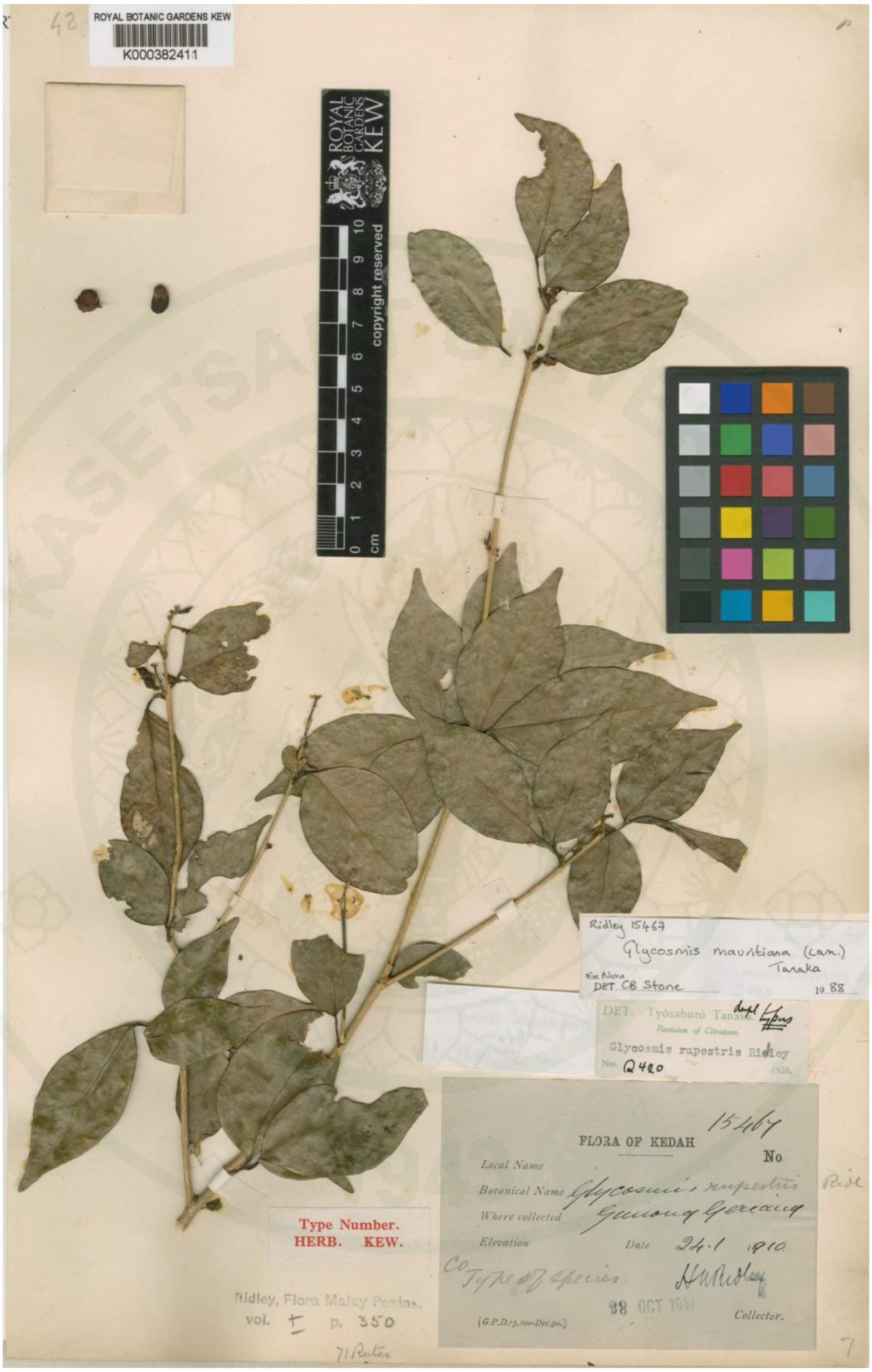
The reagent was freshly prepared before use by mixture of 0.5 ml anisaldehyde ($C_8H_8O_2$ : AR grade Fluka) in 50 ml Glacial acetic acid and 1 ml 97% sulfuric acid ($H_2SO_4$ : AR grade Fisher Scientific) following by heating on oven (Sanyo OMT) at 100-105 °C until maximal visualization of the spots.

Colorful spots will occur varies on the compound: terpene (red), sugar (grey), steroid (green), phenol (blue) and lichen (violet). Good for all things with active methylene, and for distinguishing closely-spaced spots on TLC by their color difference (Merck, 1980).

3. 10% Sodium hydroxide

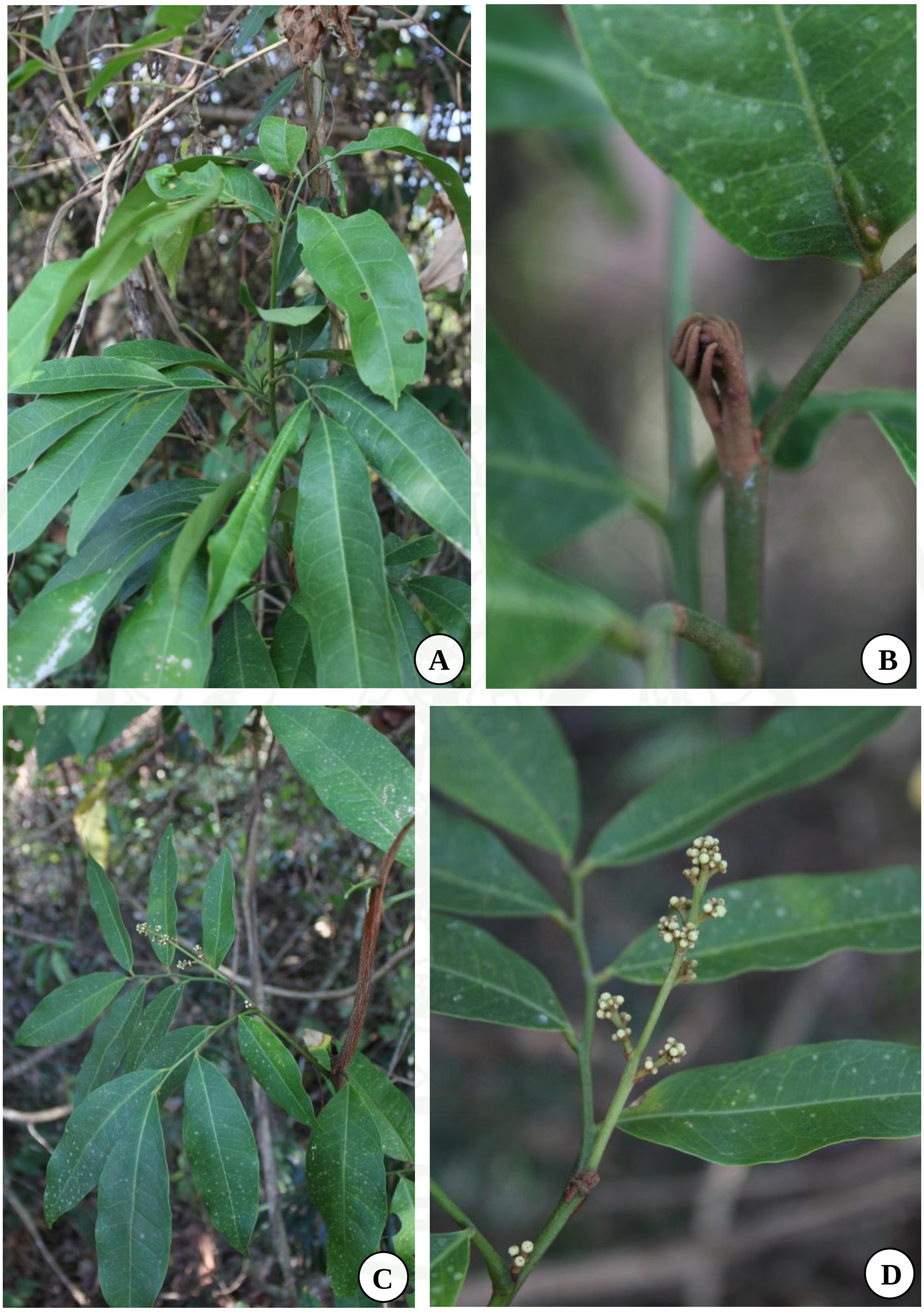
Dissolved 8 g of pellets sodium hydroxide (NaOH: AR grade Mallinckrodt) in 100 ml ethanol. After spraying, evaluate in the air. The chemical constituent was detected as yellow-green fluorescence by visualization under UV light at wavelength 365 nm (Farnsworth, 1966).


**Appendix Figure 1** Type photograph of *G. parva* Craib (From Royal Botanic Gardens, Kew)

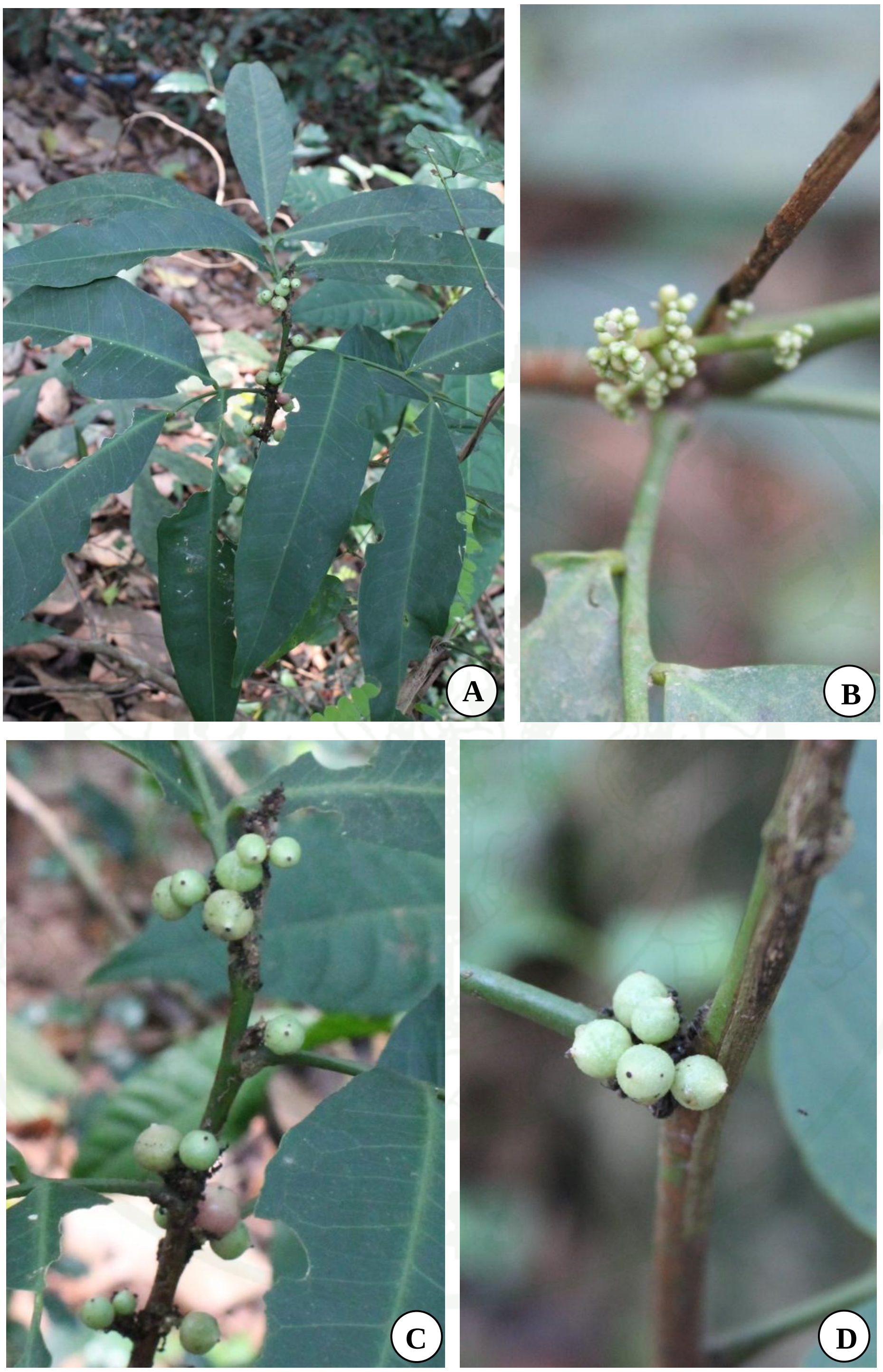



**Appendix Figure 2** Type photograph of *G. mauritiana* (Lam.) Tanaka (From Royal Botanic Gardens, Kew)

**Appendix Figure 3** *Glycosmis pentaphylla* (Trat; Trat Agroforestry Research and Training Station): A. habit; B. inflorescence; C. infructescence, photographed by W. Aiyakool




**Appendix Figure 4** *Glycosmis pentaphylla* (Chanthaburi; Krating Waterfall): A. habit; B. young leaves; C. flowering branch; D. inflorescence, photographed by W. Aiyakool

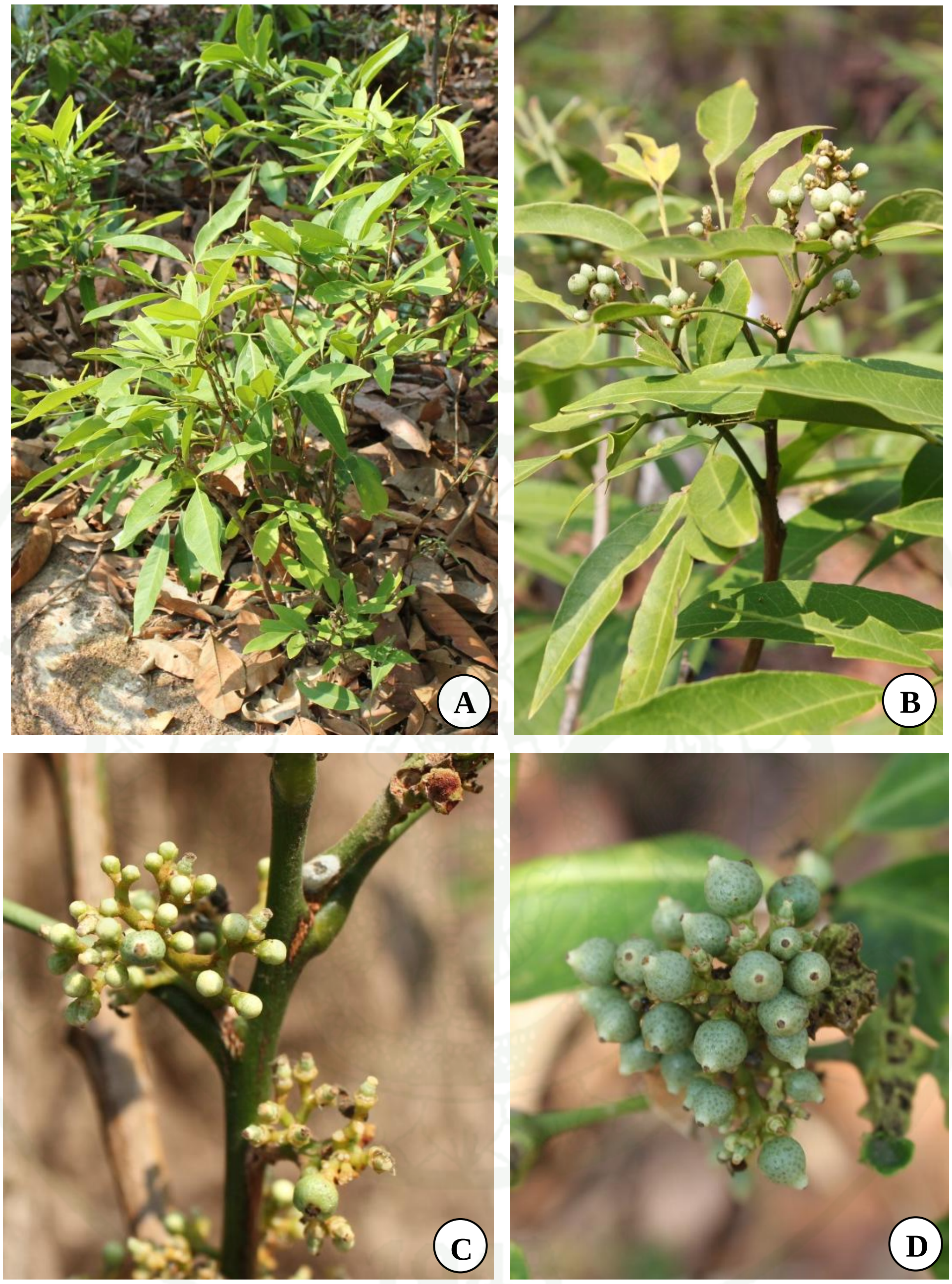



**Appendix Figure 5** *Glycosmis pentaphylla* (Rayong; Khao Chamao): A. habit; B. inflorescence; C-D. infructescence, photographed by W. Aiyakool


**Appendix Figure 6** *Glycosmis pentaphylla* (Chonburi; Kasetsart University Siracha Campus): A. habit; B. flowering branch; C. inflorescence; D. fruit, photographed by W. Aiyakool


**Appendix Figure 7** *Glycosmis pentaphylla* (Nakhon Nayok; Khao Cha-ngok): A. habit; B. flowering branch; C. inflorescence, photographed by W. Aiyakool


**Appendix Figure 8** *Glycosmis pentaphylla* (Saraburi; Sam Lan Waterfall): A. habit; B-C. inflorescence and young fruit, photographed by W. Aiyakool



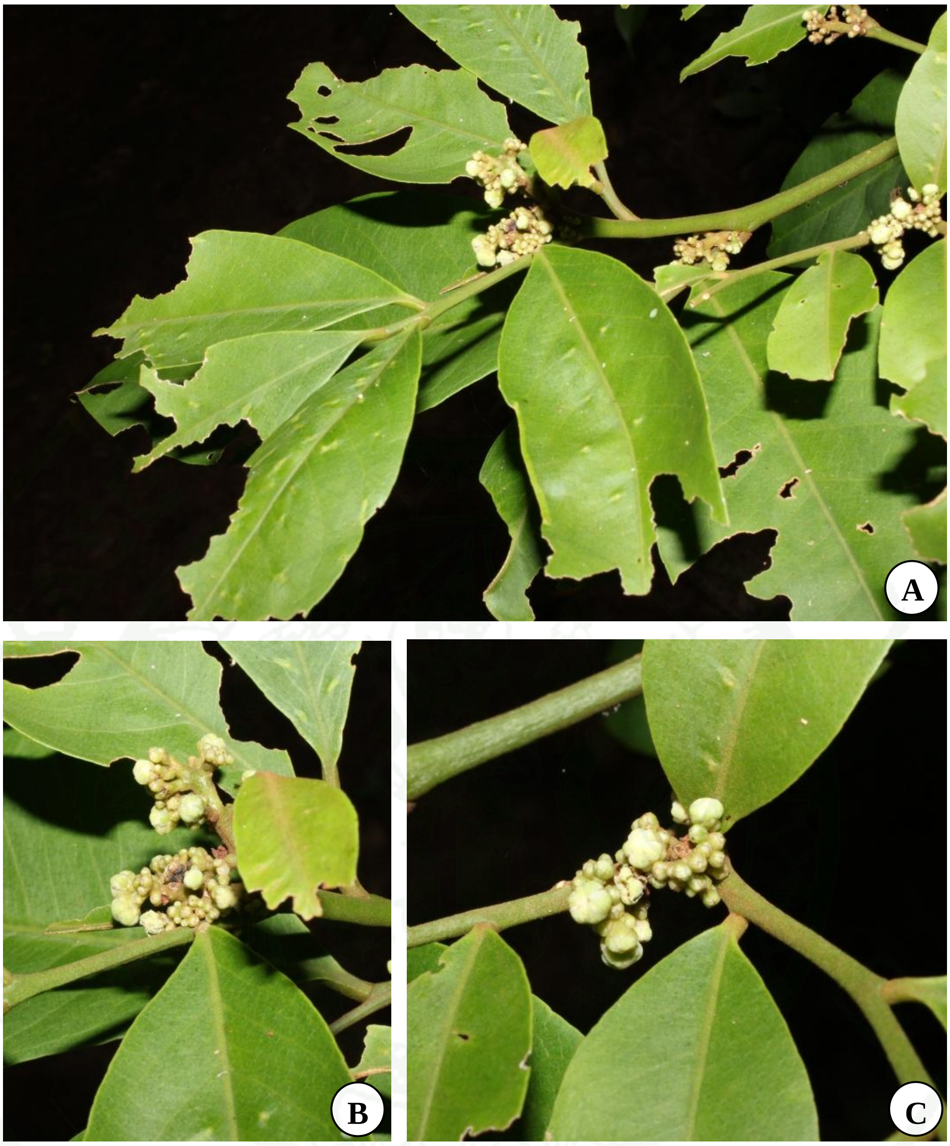

**Appendix Figure 9** *Glycosmis pentaphylla* (Nakhon Ratchasima; Haew Narok Waterfall): A. habit; B. fruiting branch; C. fruit and inflorescence; D. infructescence, photographed by W. Aiyakool


**Appendix Figure 10** *Glycosmis pentaphylla* (Bangkok): A. habit; B. young leaves; C. inflorescence; D. infructescence, photographed by W. Aiyakool


**Appendix Figure 11** *Glycosmis pentaphylla* (Phangnga; Sa Nang Manora Waterfall): A. habit; B -C. inflorescence, photographed by W. Aiyakool


**Appendix Figure 12** *Glycosmis pentaphylla* (Trang; Thung Khai Botanical Garden): A. habit; B-C. inflorescence, photographed by W. Aiyakool


**Appendix Figure 13** *Glycosmis pentaphylla* (Krabi; Sa Morakot): A. habit; B. flowering branch; C. inflorescence, photographed by W. Aiyakool




**Appendix Figure 14** *Glycosmis pentaphylla* (Nakhon Si Thammarat; Karome Waterfall): A. habit; B-C. inflorescence, photographed by W. Aiyakool


**Appendix Figure 15** *Glycosmis pentaphylla* (Satun; Tarutao Island): A. habit; B. flowering branch; C. inflorescence, photographed by W. Aiyakool

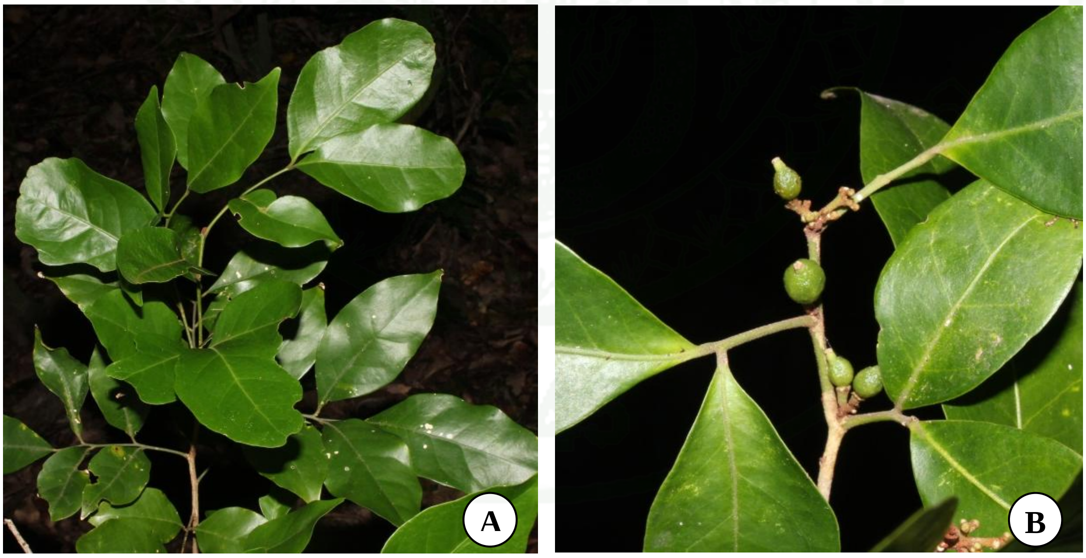

**Appendix Figure 16** *Glycosmis pentaphylla* (Songkhla; Khao Noi): A. habit; B. flowering branch; C. inflorescence, photographed by W. Aiyakool


**Appendix Figure 17** *Glycosmis parva* (Nakhon Ratchasima; Haew Narok Waterfall): A. habit; B. fruit, photographed by W. Aiyakool


**Appendix Figure 18** *Glycosmis parva* (Rayong; Khao Chamao): A. habit; B. fruit, photographed by W. Aiyakool


**Appendix Figure 19** *Glycosmis parva* (Nakhon Ratchasima; Sakaerat): A. habit; B. flower; C. fruit, photographed by W. Aiyakool


**Appendix Figure 20** *Glycosmis mauritiana* (Prachuap Khiri Khan; Laem Sala): A. habit; B. fruit, photographed by W. Aiyakool




**Appendix Figure 21** *Glycosmis mauritiana* (Prachuap Khiri Khan; Trail to Praya Nakhon Cave): A. habit; B. fruit, photographed by W. Aiyakool

## SPECIMENS EXAMINED

### Glycosmis pentaphylla

*S. Sudee, H. A. Pedersen and S. Watthana* 2595, 23 Nov. 2005, Nun Yuen, Ubon Ratchatani (QBG).

*M. Norsaengsri* 3187, 4 Dec 2007, Wang Yai, Khon Kaen (QBG).

*P. wessumritt* 106, 2 Apr 2008, Muang, Rayong (QBG).

*W. Pongamornkul* 2377, 20 June 2008, Chaing Dao, Chaig Mai (QBG).

*W. Pongamornkul* 2347, 28 May 2008, Chaing Dao, Chaig Mai (QBG).

*C. Glamwaewwong* 973, 29 Much 2005, Mae Rim Chaing Mai (QBG).

*C. Glamwaewwong* 671, 11 July 2003, Mae Rim Chaing Mai (QBG).

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*Phonchit* 427, 12 Nov 1959, Ban na, Tak (CU).

*Amorn Uboncholket* 716, 18 Feb 1960, Chaing Mai (CU).

*Wiyada N.S.*, June 1993, Bor worn ni wate Temple, Bangkok (CU).

*P. Darumas* 78, 13 Nov 2001, Pong Phu Ron, Kanchanaburi (CU).

*Herb. Trip*. 808, 8 Jan 1994, Koh Kram, Chonburi (CU).

*P. Trisarasri* 541, 2 Nov 1997, Siri Ruckhachati Garden, Nakhon Patom (CU).

*Kasin Sawatabanthu* 238, 30 April 1948, Loei (CU).

*T. Jonganurak* 139, 20 Oct 1994, Pa Klang Ao Forest Park, Prachuap Khiri Khan (CU).

*Renue Damrongtham* 3, 9 Aug 1965, Wang Takrai, Nakhon Nayok (CU).

*Pleonchit* 427, 12 Nov 1959, Ban na, Tag (CU).

*Kasin Sawatabanthu* 5, 18 August 1938, Narathivas (CU).

*P. Chantaranothai et al*. 1704, Phu Phan, Sakin Nakhon (KKU).

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*A. Verapongse* 10, 26 Mar 2005, Sunrongthab, Surin (KKU).

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*Voradol Chamchumroon* V.C. 2089, 9 Nov. 2008, Khlong Thom, Krabi (BKF, PSU)

*P. Pittisunthon* 28, 26 June 1999, Khao Pu Khao Ya National Park, Phatthalung (PSU).

*C. Phengklai* 12698, 13 Aug 2000, Khao Khieo, Chonburi (BKF).

*C. Phengklai* 11480, 12 Feb 1999, Sattahip, Chonburi (BKF).

*H.G.R.Garrett* 27, 14 April 1909, Doi Suthep, Chaing Mai (BKF).

*C. Phengklai* 13287, 5 April 2001, Kho Kut, Trat (BKF).

*J.F. Maxwell* 96-1421, 29 Oct 1996, Pae Tah, Lampang (BKF).

*J.F. Maxwell* 94-848, 31 July 1994, Pae Tah, Lampang (BKF).

*David J. Middleton, Somran Sudee, Stuart J. Davies and Jandee Hemrat* 866, 9 Aug 2002, Kaeng Krachan, Petchaburi (BKF).

*S. Phengnaren* 215, 7 Feb 1966, Lam Chabang Chonburi (BKF).

*C. Niyomtham and P. Puudja* 3296, 24 Nov 1992, Similan National Park, Phangnga (BKF).

*P. Chantaranothai, J. Parnell, D. Middleton and D. Simpson* 1051, 4 March 1993, Phu Rua National Park, Loei (BKF).

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*A.F.G. Kerr* 8140, 28 Dec 1923, Pak Thong Chai, Nakhon Ratchasima (BK).

*A.F.G. Kerr* 7265A, 19 Nov 1922, Bangkok (BK).

*Adisai* 580, 30 Oct 1963, Chaingdao, Chaing Mai (BK).

*Putt* 1269, Kaw Samut, Saratthani (BK).

*Boonsuab Chai anan* 18, 28 Aug 1954, Chaingdao, Chaing Mai (BK).

*Prayad* 642,28 Dec 1966, Ban Pan, Pisanulok (BK).

*Prayad* 1046,17 Oct 1967, Phu Kradeung, Loei (BK).

*Prayad* 445,23 July 1966, Chaing Rai (BK).

*Sakol Sutheesorn* 1425, 10 Jan 1970, Mae Chum, Chaing Rai (BK).

*D.J. Collins* 1995, 4 Nov 1927, Sriracha, Chonburi (BK).

*J.F.Maxwell* 71-630, 30 Oct 1971, Sattahip, Chonburi (BK).

*P. Sangkhachand* 1738, 3 March 1969, Khao Chong, Trang (BK).

**Glycosmis parva**

*BGO Staff* 1696, 16 Sep 1994, Phu Wieng, Khon Khaen (QBG).

*P. Piror* 1, 2 Dec 1995, Khao Aung Rue Nai, Chachoengsao (CU).

A.F.G.Kerr. 19828, 10 Nov 1930, Ban Keng, Krabin (BK).

*J.F.Maxwell* 76-682, 11 Oct 1976, Khao Kieo, Chonburi (BK).

*David J. Middleton, Somran Sudee, Stuart J. Davies and Jandee Hemrat* 895, 9 Aug 2002, Kaeng Krachan, Petchaburi (BKF).

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*C.F. van Beusekom and C. Charoenpol* 1864,28 Oct 1969, Pak Thong Chai, Nakhon Ratchasima (BKF).

*C.F. van Beusekom and R. Greesink* 3236, 22 Oct 1971, Sakaerat, Nakhon Ratchasima (BKF).

*Kai Larsen, T. Santisuk and E. Warncke* 3239, 1968, Pak Thong Chai, Nakhon Ratchasima (BKF).

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**Glycosmis mauritiana**

*Herb. Tr. (WK)* 169, 20 April 1993, Wa Kor, Prachuap Khiri Khan (CU).

*Sakol Sutheesorn* 1306, 14 July 1966, Chiya, Suratthani (BK)

*A.F.G. Kerr* 19285, 2 May 1930, Khao Hua Tak, Phatthalung (BK).

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*Put* 24805, 30 Nov 1929, Sam Roi Yawt, Prachuap Khiri Khan (BK).

*A.F.G. Kerr* 15146, 12 April 1928, Kaw Si Kaw Ha, Phatthalung (BK).

*Rabil* 330, 3 Aug 1929, Khao Wang, Trang (BK).

*Adisai* 943, 10 Nov 1964, Kuiburi, Prachuap Khiri Khan (BK).

*Sakol Sutheesorn* 2689, 22 Oct 1973, Kuiburi, Prachuap Khiri Khan (BK).

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