



Faculty of Resource Science and Technology

CHEMICAL STUDIES AND BIOLOGICAL ACTIVITIES OF *GONIOTHALAMUS TAPISOIDES*

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**Bachelor of Science with Honours
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Goniothalamus tapisoides

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This project is submitted in partial fulfilled of the requirements for the degree of
Bachelor of Science with Honours (Resource Chemistry)

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DECLARATION

No portion of the work referred to in this dissertation has been submitted in support of an application for another degree of qualification of this or any other university or institution of higher learning.

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Chemical Studies and Biological Activities of *Goniothalamus tapisoides*

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ABSTRACT

Phytochemical and biological activities studies had been performed on *Goniothalamus tapisoides*. The samples were divided into root, stem, bark and leaf and were extracted using solvent with increasing polarity. The isolation and purification process were performed using thin layer chromatography, column chromatography and preparative thin layer chromatography. Extensive column chromatography and preparative thin layer chromatography resulted in the isolation of one pure compound (63.4 mg). The pure compound has the molecular mass of 200 g/mol which corresponded to molecular formula of $C_{13}H_{12}O_2$ and melting point of $81.3^{\circ}C - 83.7^{\circ}C$. Based on spectroscopic information, melting point and comparison with literature data, the compound has been identified as goniothalamine. Goniothalamine was found in all part of the plant except the leaf. Brine shrimp toxicity test were performed on each crude extracts and also goniothalamine. The dichloromethane, chloroform and ethyl acetate crude extracts for all part of *Goniothalamus tapisoides* except leaf showed toxicity to brine shrimp with LC_{50} values between 10 ppm to 100 ppm. Goniothalamine also showed toxicity towards brine shrimp with LC_{50} values between 10 ppm to 100 ppm. Termiticidal activity test was also carried out on root and bark crude extracts. The hexane, dichloromethane, chloroform and ethyl acetate crude extract caused mortality to the termites in the concentration of 1.0% - 10.0%.

Keywords: *Goniothalamus tapisoides*, Annonaceae, goniothalamine, termiticidal activity, *Artemia salina* toxicity test

ABSTRAK

Kajian fitokimia dan aktiviti biologi telah dijalankan ke atas *Goniothalamus tapisoides*. Sampel tersebut telah dibahagikan kepada bahagian akar, batang, kulit dan daun. Bahagian-bahagian tersebut telah diekstrak menggunakan kaedah pengekstrakan dengan peningkatan kepolaran pelarut. Proses pemisahan dan penulenan telah dilakukan dengan menggunakan kaedah kromatografi lapisan nipis, kromatografi turus dan kromatografi lapisan nipis persediaan. Proses kromatografi turus dan kromatografi lapisan nipis persediaan telah berjaya memisahkan satu sebatian tulen (63.4 mg). Jisim molekul bagi sebatian tersebut adalah 200 g/mol dan bersesuaian dengan formula molekul $C_{13}H_{12}O_2$ serta takat lebur $81.3^{\circ}C - 83.7^{\circ}C$. Berdasarkan maklumat spektroskopi, takat lebur dan perbandingan dengan data perpustakaan, sebatian tersebut telah dikenalpasti sebagai goniothalamine. Goniothalamine telah dijumpai pada setiap bahagian tumbuhan tersebut kecuali daun. Ujian ketoksikan telah dijalankan ke atas semua ekstrak kasar bagi setiap bahagian dan goniothalamine. Ekstrak kasar diklorometana, kloroform dan etil asetat bagi semua bahagian kecuali bahagian daun telah menunjukkan ketoksikan terhadap anak udang dengan LC_{50} antara 10 ppm-100 ppm. Goniothalamine juga telah menunjukkan ketoksikan terhadap anak udang dengan LC_{50} antara 10 ppm-100 ppm. Aktiviti termitisidal terhadap anai-anai juga telah dijalankan bagi ekstrak kasar bagi bahagian akar dan kulit. Ekstrak kasar heksana, diklorometana, kloroform dan etil asetat didapati menyebabkan kematian anai-anai pada kepekatan 1.0% -10.0%.

Kata kunci: *Goniothalamus tapisoides*, Annonaceae, goniothalamine, aktiviti termitisidal, ujian ketoksikan *Artemia salina*

CHAPTER 1

INTRODUCTION

1.1 General

The genus *Goniothalamus* is a member of Annonaceae family consist about 115 species of shrubs and trees distributed in the tropic and subtropics especially in the Asia region (Hisham *et al.*, 2000). Some *Goniothalamus* species such as *Goniothalamus giganteus*, *Goniothalamus gardneri*, *Goniothalamus griffithii*, *Goniothalamus borneensis* and *Goniothalamus cardiopetalus* (Chen *et al.*, 1998; Alali *et al.*, 1998a; Alali *et al.*, 1998b; Seidel *et al.*, 1999; Hisyam *et al.*, 2003) are widely distributed in the Asian region.

Several *Goniothalamus* species such as *Goniothalamus malayanus* and *Goniothalamus borneensis* which are well known in Malaysia had been studied and various biologically active compounds such as acetogenins, alkaloids, styryl lactones and flavonoids have been isolated (Seidel *et al.*, 1999). The important major class of secondary metabolites from *Goniothalamus* species are acetogenins and the styryl lactones (Fátima *et al.*, 2005). About 250 bioactive acetogenins were isolated from the Annonaceae family which includes the *Annona*, *Asimina*, *Rollinia*, *Uvaria* and *Goniothalamus* genus (Seidel *et al.*, 1999).

Various *Goniothalamus* species are used as folk medicine in some countries (Surivet and Vatéle, 1999). In southern Taiwan, *Goniothalamus amuyon* seeds had been used for treating edema and rheumatism (Wu *et al.*, 1991). In Sabah, *Goniothalamus borneensis* is used by the natives as mosquito repellent (Cao *et al.*, 1998). *Goniothalamus*

scortechinii, a shrub which can be found in the highland area of is used as past parturition aids and also to cure jaundice (Zakaria and Mohd, 1992). Due to the uses in traditional medicine, various *Goniothalamus* species had been studied to determine the biological active compounds and its potency to produce new drugs for future application.

1.2 Objectives

Goniothalamus spp. are widely distributed in Malaysia and many species in this genera are not scientifically studied. There were limited published data on this genus. These genera have shown interesting biological activities which have potential in the pharmacological industries in order to produce new potent drugs. To the best of our knowledge, *Goniothalamus tapisoides* has not been scientifically studied. Therefore the objectives of this research are:

- a) to extract, isolate and purify the secondary metabolites from *Goniothalamus tapisoides*.
- b) to elucidate the structure of the pure compounds isolated from *Goniothalamus tapisoides* using various spectroscopic methods.
- c) to evaluate the toxicity of the crude extract, fractions, semi-pure compounds and pure compounds isolated from *Goniothalamus tapisoides* on brine shrimps, *Artemia salina*.
- d) to determine the termiticidal activity of crude extracts of *Goniothalamus tapisoides*.

CHAPTER 2

LITERATURE REVIEW

2.1 Annonaceae family

Extensive research has been performed on the Annonaceae family. Various secondary metabolites had been isolated from this family especially for *Annona*, *Asimina*, *Rollinia*, *Uvaria* and *Goniothalamus* genus (Seidel *et al.*, 1999). The secondary metabolites that have been isolated from this family can be classified as Annonaceous acetogenins, pyrenes, alkaloids, flavonoids and styryl lactones which showed interesting biological activities.

Members in the Annonaceae family are widely used in traditional medicine to cure various diseases. For example, *Artabotrys hexapetalus* are used in the Chinese traditional folk medicine (Li *et al.*, 1997). The roots are used to cure malaria while the fruits are used in treating scrofula. Phytochemical studies on this species afforded various sesquiterpenes and alkaloids which possess antimalarial and antitumoural activities respectively (Li *et al.*, 1997). *Uvaria lucida* had been used in Tanzania as remedies for malaria and stomach disorder (Achenbach *et al.*, 1997).

2.2 Secondary Metabolites from Annonaceae Family

Various secondary metabolites have been characterized from the Annonaceae family. Alkaloids have been focused as important secondary metabolites from the plants of Annonaceae family until the discovery of Annonaceous acetogenins. Annonaceous

acetogenins (Figure 2.1) belongs to a new class of natural products based on bistetrahydrofuranoid fatty acid lactones (Rahman, 1996). This new class of natural products displayed an interesting antitumor activity.

These secondary metabolites are produced by polyketide origin through the acetyl coenzyme A elongation process. In 1984, a related compound was isolated and identified as rollicin which gives interesting cytotoxic activity (Rahman, 1996). It had been described and the name of acetogenins was proposed for this kind of secondary metabolites.

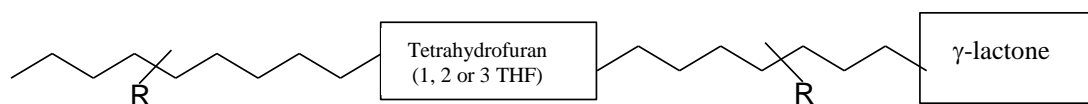


Figure 2.1: Annonaceous acetogenin

Acetogenin molecules possess 35-37 carbon atoms. In the long alkyl chain, there are oxygenated functional groups for examples hydroxyl, acetoxy, ketone, etc. and/or double bonds and one to three tetrahydrofuran with the presence of γ -butyrolactone at the end of the molecule. Acetogenins possessed many stereogenic centres due to the presence of the functional groups (Rahman, 1996).

Annonaceous acetogenins can be classified into four main types that are A, B, C and D as a function of the number and position of tetrahydrofuran rings presence. Type A is characterized by the presence of one tetrahydrofuran ring (Myint *et al.*, 1991). Type B having two adjacent tetrahydrofuran rings (Duret *et al.*, 1994). Type C is characterized by tetrahydrofuran rings that are separated by four carbon atoms (Cortes *et al.*, 1991) and type

D is characterized by three contiguous tetrahydrofuran rings (Rahman, 1996) as shown in Figure 2.2.

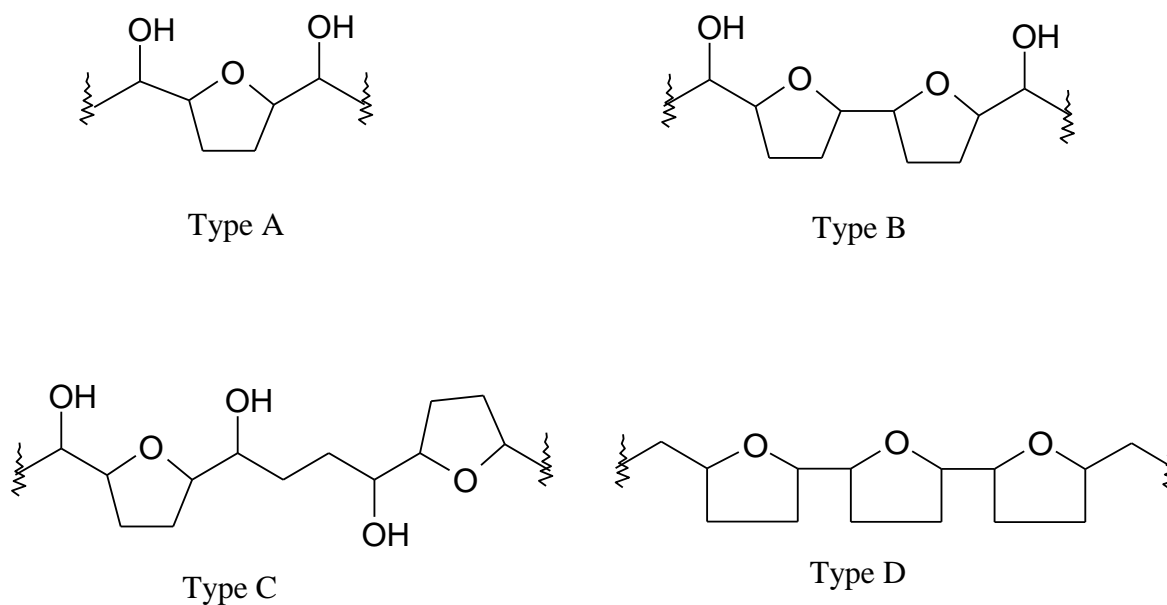


Figure 2.2: Acetogenins types structures

The main classes are subdivided into 3 subtypes that are 1, 2, and 3. These subclasses are characterized by the function of the nature of the γ -butyrolactone. First subclass is characterized with the present of α,β -unsaturated γ -methyl- γ -lactone, the second subtype with the presence of α -acetyl- γ -butyrolactone and the third subtype is characterized with the presence of β -hydroxy- γ -methyl- γ -lactone (Rahman, 1996) as shown in Figure 2.3.

About 128 different linear, epoxy and mono-tetrahydrofuran had been characterized from the Annonaceae family (Polo *et al.*, 1995). The linear and epoxy acetogenins belongs to a class biogenetic precursor of the tetrahydrofuran acetogenins.

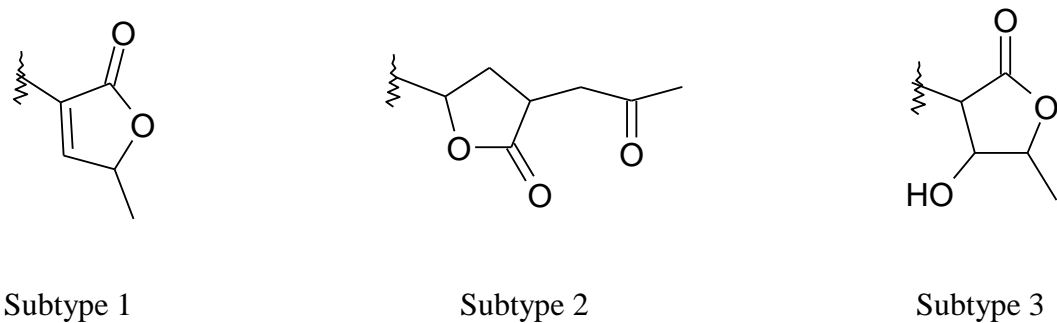
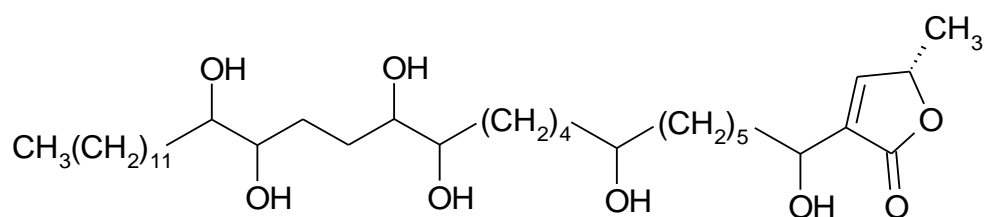
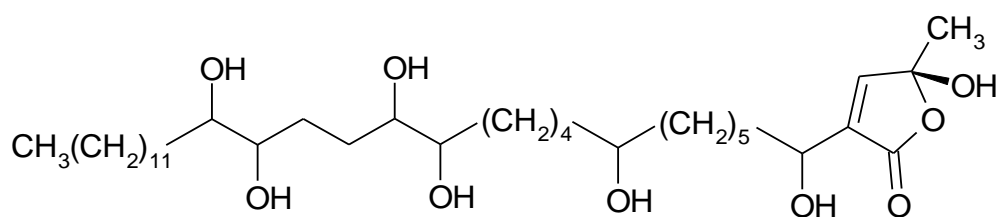
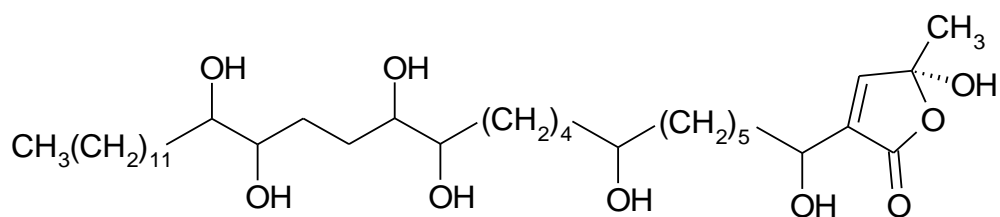
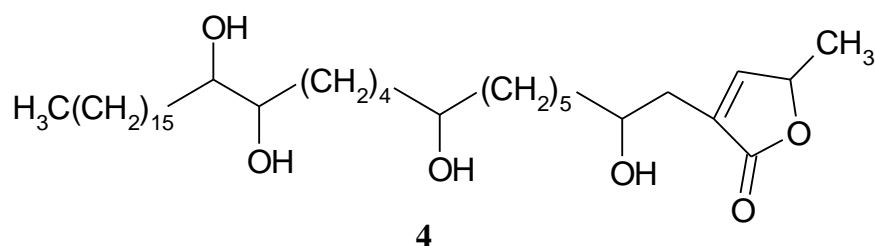


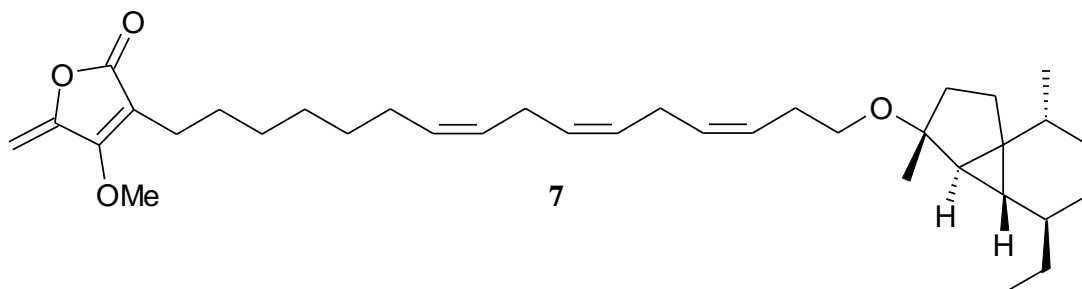
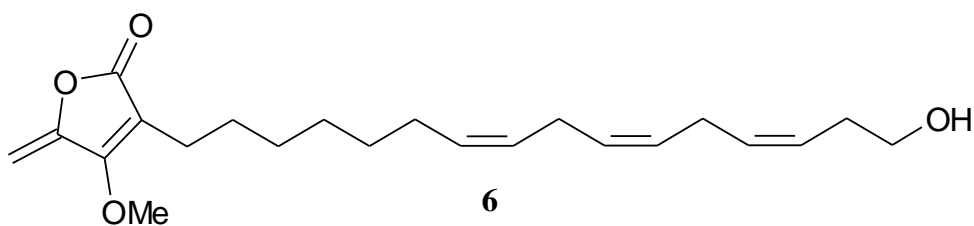
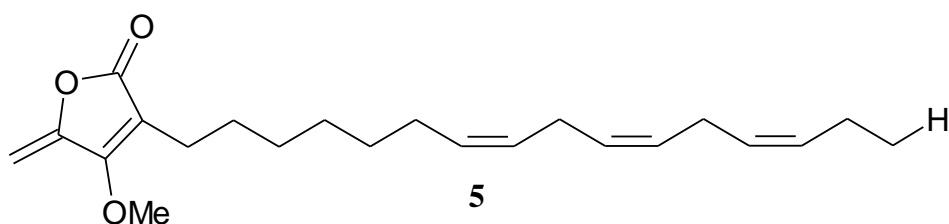
Figure 2.3: Acetogenins subtypes structures

In 1997, four linear acetogenins had been isolated from *Goniothalamus donnaiesis*. The compounds were characterized as donhepocin (**1**), 34-*epi*-donhepocin (**2**), donhexocin (**3**) and donbutocin (**4**) (Jiang *et al.*, 1998).



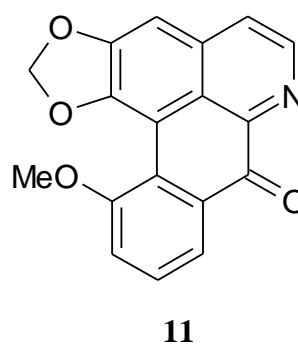
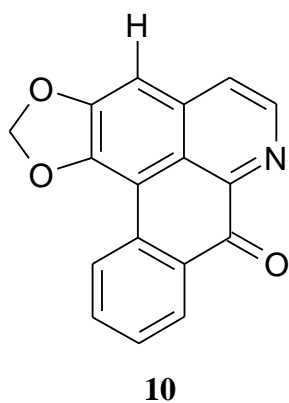
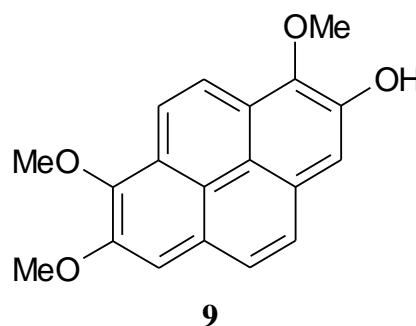
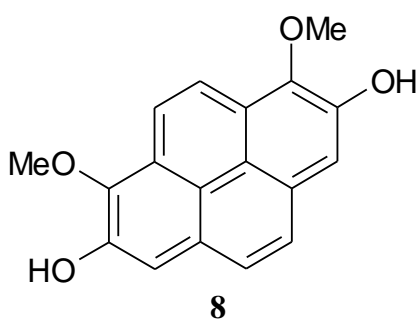


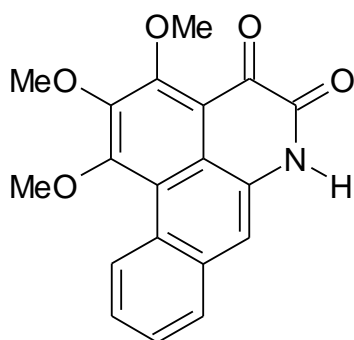
The dichloromethane extracts of *Artabotrys hexapetalus* resulted in the isolation of three β -methoxy- γ -methylene- α,β -unsaturated- γ -butyrolactones (Wong and Brown, 2002). All the compounds were derived from the C_{18} unsaturated fatty acid by biosynthetic route (Wong and Brown, 2002). The first compound was characterised as artapetalin A (**5**) which have the molecular formula of $C_{22}H_{32}O_3$ with five double bonds and a ring (Wong and Brown, 2002). Artapetalins B (**6**) and C (**7**) had been identified as the 21-hydroxyl and 21-(4-*epi*-cubebol) which were the derivatives of **5**.



Genus *Uvaria* consist about 150 species and only about twenty of these species has been studied and the secondary metabolites characterized (Fleischer *et al.*, 1998). Two pyrenes has been isolated from the chloroform extract of the stem bark of *Uvaria lucida* (Achenbach *et al.*, 1997). The pyrenes were isolated together with some other known compounds. Two of the oxygenated pyrenes compounds were identified as 2,7-dihydroxy-1,8-dimethoxypyrene (**8**) and 2-hydroxy-1,7,8-trimethoxypyrene (**9**) (Achenbach *et al.*, 1997).

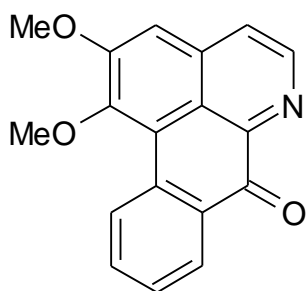
Phytochemical studies on *Pseuduvaria rugosa* which belong to the Annonaceae family resulted the isolation of alkaloid such as liriodenine (**10**), oxoputerine (**11**), Ouregidione (**12**), methoxycepharadione (**13**) and rugonasine (**14**) (Jamaludin and Hadi, 1999).



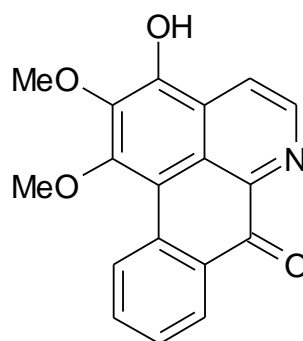


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Alkaloids such as lysicamine (**15**), liriodenine (**10**) and isomoschatoline (**16**) were characterized from *Uvaria mocoli* (Fleischer *et al.*, 1998).



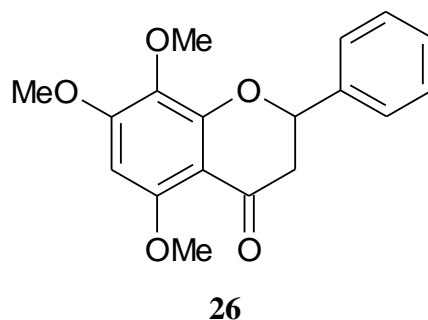
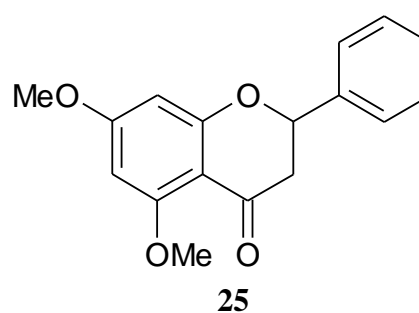
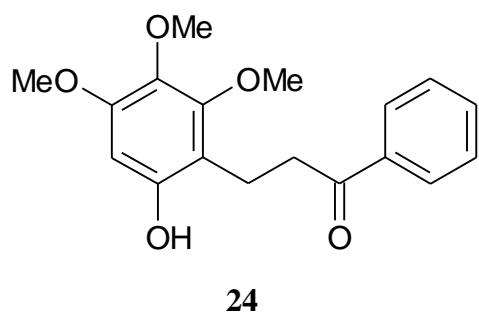
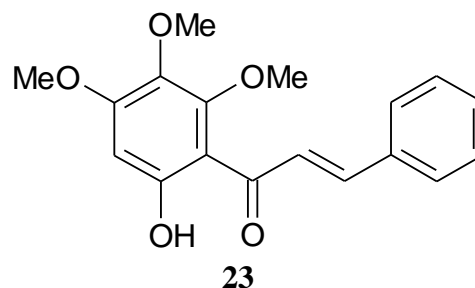
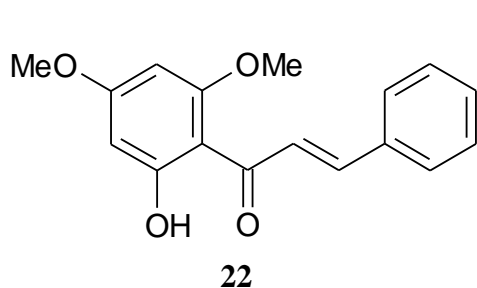
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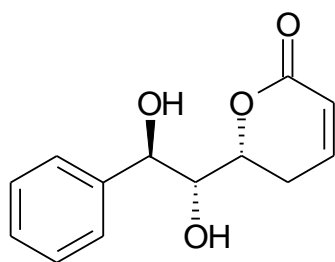
Flavonoids had been isolated from many members in the Annonaceae family for examples *Uvaria* spp. and *Artabotrys* spp. (Achenbach *et al.*, 1997; Li *et al.*, 1997; Fleischer *et al.*, 1998). Two flavonol glycosides which were identified as arapetaloside A (**17**) and B (**18**) were isolated from the leaves of *Artabotrys hexapetalus*. Taxifolin (**19**), apigenin-7-*O*-apiosyl (1→2) glucoside (**20**) and glucoluteolin (**21**) were also isolated from the similar species. Studies on *Uvaria mocoli* afforded five flavonoids which were identified as 2'-hydroxy-4',6'-dimethoxychalcone (**22**), 2'-hydroxy-4',5',6'-

trimethoxychalcone (**23**), 2-hydroxy-4,5,6-trimethoxydihydrochalcone (**24**), 5,7-dimethoxyflavanone (**25**) and 5,7,8-trimethoxyflavanone (**26**) (Fleischer *et al.*, 1998).

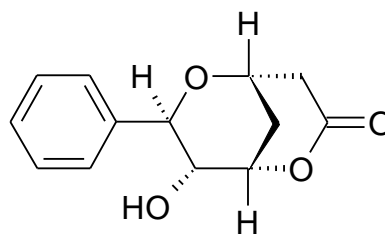


Styryl lactones are found in various *Goniothalamus* spp.. Styryl lactones are a series of natural products with moderate to significant biological activities (Chen *et al.*, 2004). More than twenty different styryl lactones had been isolated and identified from fungi and plants (Chen *et al.*, 2004). Styryl lactones such as 7-*epi*-goniodiol (**27**) and

leiocarpin A (**28**) were isolated from the ethanol extract of *Goniothalamus leiocarpus* (Chen *et al.*, 2004).



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2.3 *Goniothalamus* spp.

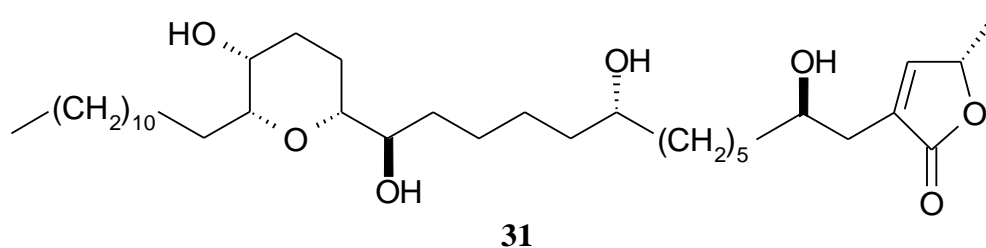
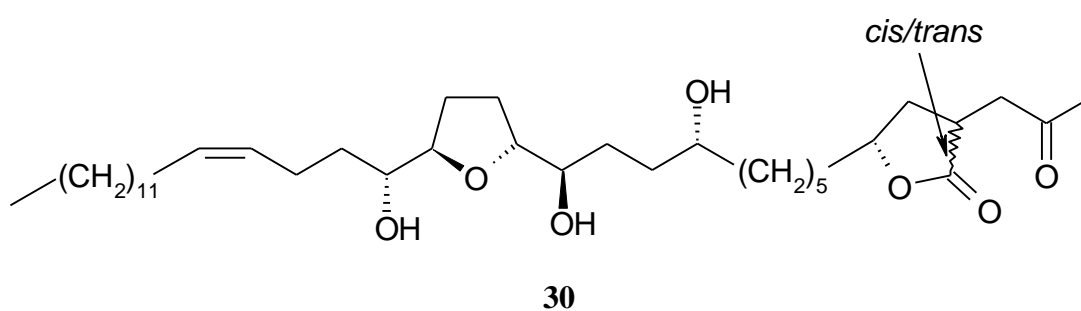
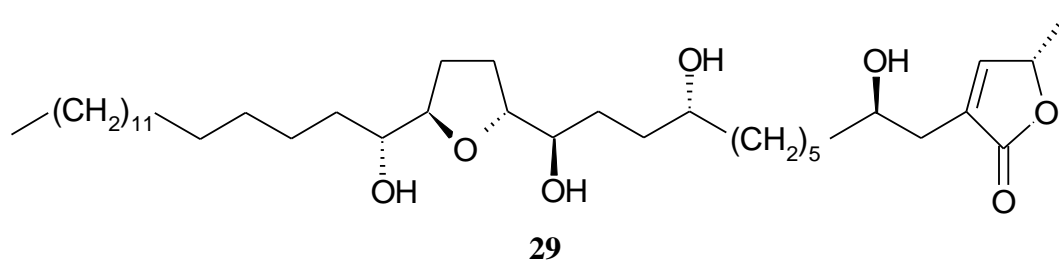
About 155 species of *Goniothalamus* are found around the world (Hisham *et al.*, 2000). Many species of *Goniothalamus* for examples *G. scortechinii*, *G. donnaiensis*, *G. giganteus*, *G. gardnerii*, *G. cardiopetalus* and others can be found easily. Various secondary metabolites have been isolated and characterized from this genus. Annonaceous acetogenins, styryl lactones and alkaloids are the major classes of secondary metabolites reported in this genus.

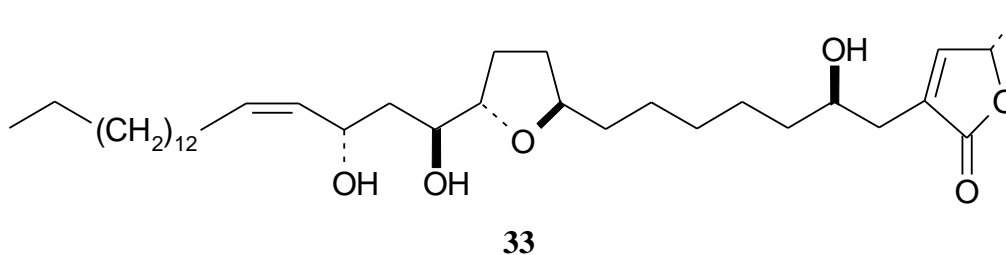
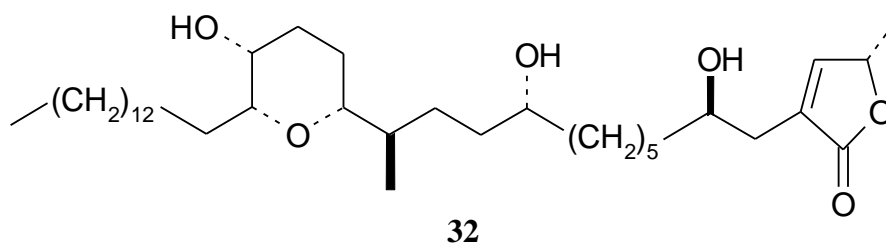
2.4 Secondary Metabolites from *Goniothalamus* spp.

Annonaceous acetogenins with γ -lactone groups had been isolated from many *Goniothalamus* spp.. Several researcher reported the isolation and characterization of various Annonaceous acetogenins (Alali *et al.*, 1998a, 1998b; Chen *et al.*, 1998; Jiang *et al.*, 1998).

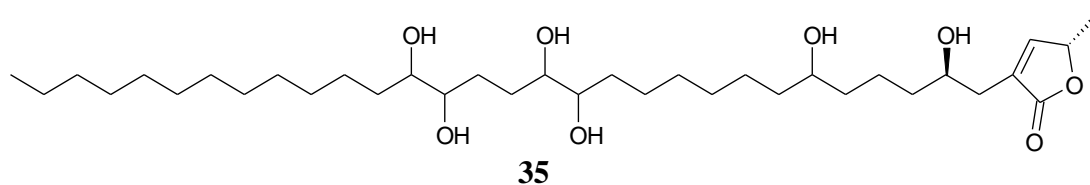
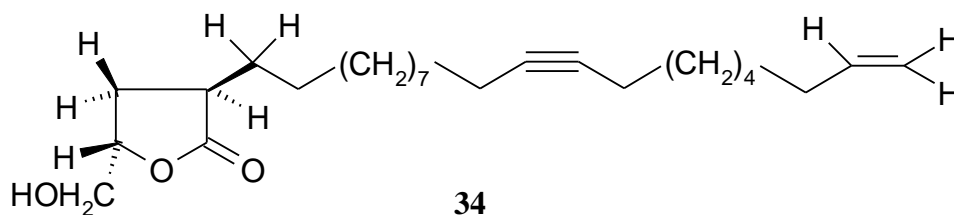
Four acetogenins was isolated from the roots of *Goniothalamus donnaiensis*. The isolated compounds had been characterized as donhepocin (**1**), 34-*epi*-donhepocin (**2**), donhexocin (**3**) and donbutocin (**4**) (Jiang *et al.*, 1998). Donhepocin (**1**) and 34-*epi*-donhepocin (**2**) had been identified as an epimeric pair which contained γ -hydroxymethyl- γ -lactone (Jiang *et al.*, 1998).

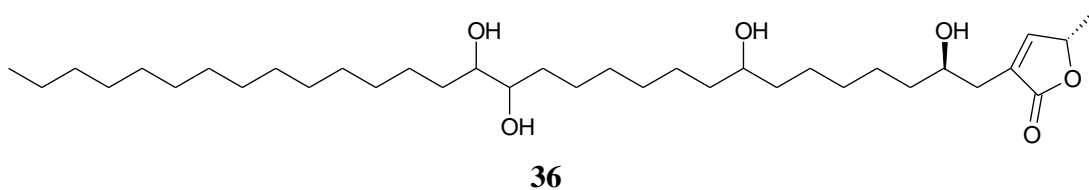
Several Annonaceous acetogenins had been isolated from the bark of *Goniothalamus giganteus* (Alali *et al.*, 1998a; 1998b). Some of the compounds identified include gionotetracin (**29**), a mixture of (2,4-*cis* and *trans*)-gonioneninone (**30**), pyranicin (**31**), pyragonicin (**32**) and gionotrioinin (**33**) (Alali *et al.*, 1998a; 1998b).





The aerial and roots of *Goniothalamus gardneri* afforded some Annonaceous acetogenins (Chen *et al.*, 1998; Seidel *et al.*, 1999). Goniothalamusin (**34**) was isolated in the form of amorphous solid (Seidel *et al.*, 1999) and identified as a linear acetylenic and olefinic acetogenin with a C₂₅ skeleton (Seidel *et al.*, 1999). The roots of *Goniothalamus gardneri* afforded two compounds identified as gardnerilin A (**35**) and gardnerilin B (**36**) (Chen *et al.*, 1998).

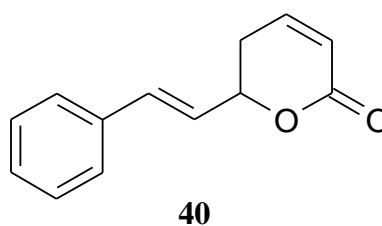
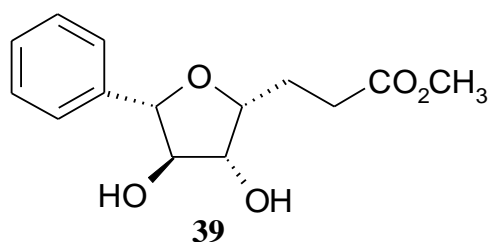
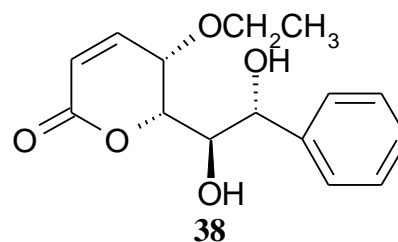
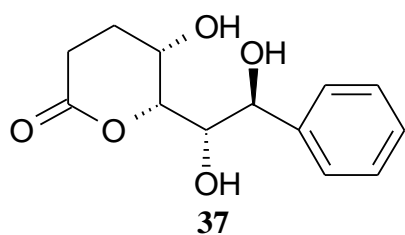


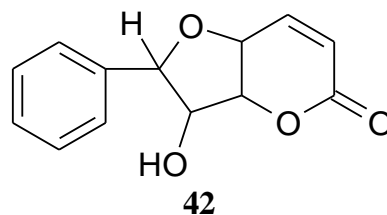
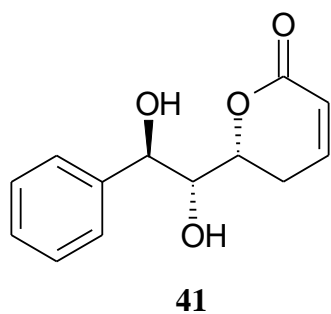


Styryl lactones or styryl pyrones are common secondary metabolites found in the *Goniothalamus* spp.. Styryl lactones isolated from several *Goniothalamus* spp. are given in Table 2.1.

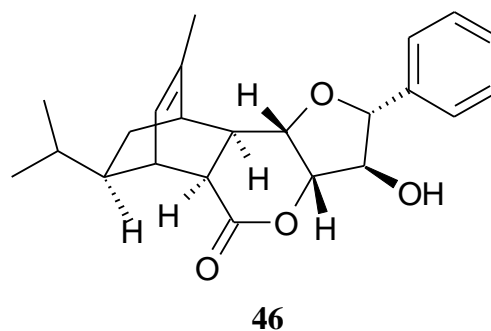
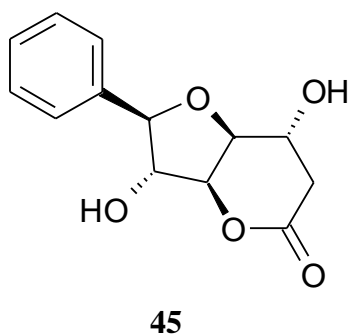
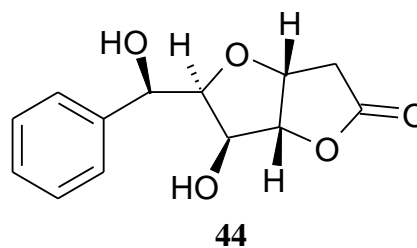
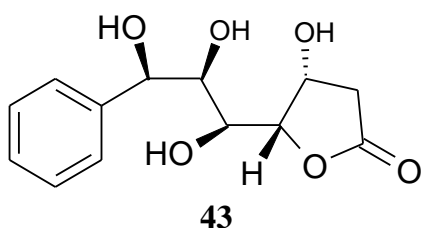
Table 2.1: Styryl lactones isolated from various *Goniothalamus* spp.

Plant species	Part of the plant used	Styryl lactones	References
<i>Goniothalamus arvensis</i>	Stem bark	(+)-garvensintriol (37), (-)-etharvendiol (38)	Bermejo <i>et al.</i> , 1997
<i>Goniothalamus borneensis</i>	Bark	Goniothalesdiol (39),	Cao <i>et al.</i> , 1998
<i>Goniothalamus macrophyllus</i>	Roots, stems and leaves	Goniothalamine (40)	Wattanapiromsakul <i>et al.</i> , 2005
<i>Goniothalamus griffithii</i>	Stem and branch	Goniothalamine (40), goniodiol (41), altholactone (42)	Tian <i>et al.</i> , 2006





Phytochemical studies on *Goniothalamus cardiopetalus* also afforded some styryl lactones especially cardiobutanolide (**43**), goniofufurone (**44**), goniofupyrone (**45**) and cardiopetalolactone (**46**) (Hisham *et al.*, 2000; Hisham *et al.*, 2003).



2.5 Biological Activities of Secondary Metabolites from *Goniothalamus* spp.

Secondary metabolites isolated from *Goniothalamus* spp. showed interesting biological activities. Several studies were performed on Annonaceous acetogenins in the search of new biological active compounds. For examples, extensive studies on *Goniothalamus donnaiensis* resulted in the isolation of donhepocin (**1**), 34-*epi*-donhepocin