



Faculty of Resource Science and Technology

PHYTOCHEMICAL STUDIES AND BIOLOGICAL ACTIVITIES OF
Goniothalamus malayanus

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Bachelor of Science with Honours
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This Final Year Project is submitted in partial fulfillment of the requirement for the degree of

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DECLARATION

No portion of the work referred 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.

Siti Awanis Binti Abdullah

Program of Resource Chemistry

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak

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Phytochemical studies and biological activities of *Goniothalamus malayanus*

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ABSTRACT

The Phytochemical studies and biological activities of *Goniothalamus malayanus* have been conducted. The roots and stem barks of *Goniothalamus malayanus* were extracted with methanol and yield 13.08 g (1.31%) and 12.85 (0.71%) crude extract respectively. The methanol crude extract for both parts were partitioned using solvent with increasing polarity in the order of hexane, dichloromethane, chloroform and ethyl acetate. Ethyl acetate crude extract of the roots and DCM crude extract of the stem barks were subjected to column chromatography. For purification process, extensive column chromatography of the extracts gave one pure compound from the roots which was labeled as GMEtOAc7 and one semi pure compound from the stem barks and labeled as GMDCM6.7. Both compounds were subjected to various spectroscopic analyses such as IR, MS and NMR to elucidate the structure. Based on spectroscopic information, the compound isolated from both fractions was identified as goniothalamine. Ethyl acetate crude extract from the roots was toxic against *Artemia salina* which gave LC₅₀ of 33.89 µg/mL while for the stem barks sample, dichloromethane crude extract was toxic against *Artemia salina* which gave LC₅₀ value of 50.12 µg/mL. Ethyl acetate crude extract from roots of *Goniothalamus malayanus* was the most toxic against *Coptotermes sp.* that showed LC₅₀ value of 1.50%. While for the stem barks of *Goniothalamus malayanus*, DCM crude extract was the most toxic against *Coptotermes sp.* that gave LC₅₀ value of 0.17%.

Keywords: *Artemia salina*, Biological activities, *Coptotermes sp.*, *Goniothalamus malayanus*, Phytochemical studies.

ABSTRAK

Kajian fitokimia dan aktiviti biologi ke atas *Goniothalamus malayanus* telah dijalankan. Akar dan kulit bagi *Goniothalamus malayanus* telah diekstrak dengan menggunakan methanol dan menghasilkan 13.08 g (1.31%) dan 12.85 g (0.71%) ekstrak kasar masing-masing. Ekstrak kasar methanol untuk kedua bahagian telah dipisahkan dengan pelarut mengikut pertambahan kekutuban dalam urutan heksana, diklorometana, klorofom dan etil asetat. Ekstrak kasar etil asetat bagi bahagian akar dan ekstrak kasar diklorometana bagi bahagian kulit telah digunakan untuk proses kolum kromatografi. Untuk proses penulenan, kaedah kolum kromatografi secara meluas bagi ekstrak kasar menghasilkan satu komponen yang tulen daripada bahagian akar yang mana di tanda sebagai GMEtOAc7 dan satu komponen yang tidak tulen daripada bahagian kulit yang mana di tanda sebagai GMDCM6.7. Kedua-dua komponen telah dipilih untuk pelbagai analisis spektroskopi seperti IR, MS dan NMR untuk mengenal pasti struktur komponen tersebut. Berdasarkan maklumat spektroskopi, komponen yang telah dipisahkan daripada kedua-dua fraksi telah dikenal pasti sebagai goniothalamine. Ekstrak kasar etil asetat daripada bahagian akar adalah toksik terhadap *Artemia salina* dan memberikan nilai LC_{50} ialah 33.89 $\mu\text{g/mL}$. Manakala untuk bahagian kulit, ekstrak kasar diklorometana adalah toksik terhadap *Artemia salina* yang menunjukkan nilai LC_{50} adalah 50.12 $\mu\text{g/mL}$. Ekstrak kasar etil asetat daripada bahagian akar *Goniothalamus malayanus* adalah toksik terhadap *Coptotermes* sp. yang menunjukkan nilai LC_{50} adalah 1.59%. Manakala untuk bahagian kulit, ekstrak kasar diklorometana adalah toksik terhadap *Coptotermes* sp. yang menunjukkan nilai LC_{50} adalah 0.17%.

Kata kunci: Aktiviti biologi, *Artemia salina*, *Coptotermes* sp., *Goniothalamus malayanus*, Kajian fitokimia.

CHAPTER 1

INTRODUCTION

1.1 Background of study

This study focused on the genus *Goniothalamus* which is classified in the Annonaceae family. *Goniothalamus* is a genus of shrubs and aromatic trees with approximately 160 species distributed in South Eastern Asia and throughout Malaysia (Goh *et al.*, 1995) and only 22 species of this genus had been investigated so far (Wiert, 2007). The family of Annonaceae is a large primitive and is presented by large group of about 120 genera and over 2000 species of trees, shrubs or climbers mostly distributed in the tropical and subtropical lowland forest of Africa, South America, Southeast Asia and Australia (Wiert, 2000).

Annonaceae are well known and can provide specific substance with interesting biological activity (Fasihuddin and Hasmah, 1993). Some example of plant in Annonaceae family are *Annona squamosa* that are widely used by people in peninsular India as the medicine for vomiting, diarrhea, dysentery and vertigo (Alali *et al.*, 1999) and *Uvaria grandifolia* used to treat stomach-ache and as a postpartum medicine (Wiert, 2000). The genus of *Goniothalamus* is widely used in traditional medicines by natives especially for abortion and post partum treatment. *Goniothalamus* species has shown significant cytotoxic activities against several human tumor cancer cell lines (Hisham *et al.*, 2000). Decoctions of *Goniothalamus macrophyllus* and *Goniothalamus scortechinii* are used as a post partum protective remedy while the roots of *Goniothalamus tapis* and *Goniothalamus giganteus* are used for abortion during early month of pregnancy (Wiert, 2007).

Secondary metabolites are those compound produced by using primary metabolites and are not directly essentials for the basis photosynthesis and respiration metabolism (Theis and Lerdau, 2003). Studies on *Goniothalamus* species have resulted in the isolation of various compounds such as isoquinoline-derived alkaloids (Omar *et al.*, 1992), styryl-lactones (Bermejo *et al.*, 1998) and acetogenins (Zafra-Polo *et al.*, 1998) with significant antimicrobial, cytotoxic and insecticidal activities. Antitumor, teratogenic and embryotoxic activities have also been reported in other isolated compounds from *Goniothalamus* species (Sam *et al.*, 1987; Hasan *et al.*, 1994).

1.2 Objectives

The objective of this research is to perform phytochemical studies and biological activities on *Goniothalamus* species. The specific objectives are:

- a) to extract the chemical compounds from the roots and stem barks of *Goniothalamus malayanus*.
- b) to elucidate the chemical structures of the pure compounds isolated from *Goniothalamus malayanus* using various spectroscopic information.
- c) to determine the biological activities of the crude extract, partitions and toxicity of pure compounds isolated from *Goniothalamus malayanus* especially against brine shrimp *Artemiasalina* and termiticidal activity against *Coptotermes* sp.

CHAPTER 2

LITERATURE REVIEW

2.1 Annonaceae Family

Annonaceae which also known as custard apple family mostly grows in the rainforest and it is a large family of tropical plant (Alali *et al.*, 1999). Some example of Annonaceae genera are *Afroguatteria*, *Alphonsea*, *Ambavia* and others. All the genera can be found mostly in North America, Middle America, South America, Africa, Asia Southeastern, Australia, and Oceania. Annonaceae family occurs primarily as the aromatic trees, shrubs or climbers (Alali *et al.*, 1999).

Most of the Annonaceae family has been used traditionally by various communities for medicinal purposes. Table 2.1 shows the medicinal uses of some species of Annonaceae family.

Table 2.1: Medicinal Uses of Some Annonaceae Family (Wiart, 2000)

Species	Medicinal Purposes
<i>Annona muricata</i>	Leaves used as insecticide, used externally for rheumatism, cough and fever and also applied to scabies and skin diseases Green barks and leaves are used to wash ulcer and wound
<i>Cananga orodata</i>	Barks is used for scabies Flower is used for malaria and a paste of flower is applied to chest to treat asthma Essential oil makes an external remedy for cephalgia, ophtalmia and gout
<i>Cyathostemma argenteum</i>	Used to treat cancer Barks are used as antispasmodic
<i>Desmos chinensis</i>	Roots are used for treating diarrhea, dysentery, vertigo and post partum medicine
<i>Fissistigma</i> sp.	Root and flower are used to treat stomach-ache
<i>Polyalthia cauliflora</i>	Paste of leaves are applied to treat skin diseases
<i>Uvaria grandifolia</i>	Used to treat stomach-ache and as a postpartum medicine

2.2 *Goniothalamus* species

2.2.1 General

Goniothalamus species are widely distributed in Malaysia. Table 2.2 shows the distribution of *Goniothalamus* species in Malaysia.

Table 2.2: The Distribution of *Goniothalamus* species in Malaysia (Mat Salleh, 1993)

Species	Location
<i>G. andersonii</i>	Sarawak
<i>G. borneensis</i>	Sarawak
<i>G. curtisii</i>	Kelantan, Perak, Selangor
<i>G. giganteus</i>	Peninsular Malaysia, Sarawak
<i>G. malayanus</i>	Peninsular Malaysia (except Perlis and Malacca), Sarawak, Sabah
<i>G. macrophyllus</i>	Peninsular Malaysia (except Perlis), Sarawak, Sabah
<i>G. ridleyi</i>	Peninsular Malaysia, Sarawak
<i>G. sinclairianus</i>	Sarawak
<i>G. tapis</i>	Penang, Perak, Johor, Sarawak
<i>G. umbrosus</i>	Peninsular Malaysia
<i>G. uravoides</i>	Kedah, Perak, Johor, Sarawak
<i>G. velutinus</i>	Sarawak, Sabah

Goniothalamus species are widely employed in traditional medicine, alone or as part of herbal mixtures, as post-partum protective remedies, abortifacient and insects repellents (Perry and Metzger, 1980). Some of the members in the Annonaceae family have been investigated and various biological activities have been observed and recorded. Compounds isolated from this family are highly effective to block mitochondrial respiratory chain that is the bioenergetic process, thus they might be exploited for antitumor therapy, biomedical research, and agrochemical pest control, like other respiratory chain inhibitors (Peris *et al.*, 2000).

2.2.2 Important of *Goniothalamus* species in Traditional Medicine

Goniothalamus species are widely used in traditional medicine by the local communities in Malaysia. *Goniothalamus* species are widely used in traditional medicine such as *Goniothalamus malayanus* which is used for abortion, to treat fever, measles and rheumatism (Mat-Salleh and Ahmad, 1989; Latiff, 1991) and *Goniothalamus tapis* used to treat stomachache, diarrhea and for abortion (Latiff, 1991; Wiart, 2000; Ahmad *et al.*, 2001). *Goniothalamus* species provided a number of medicinal uses as given in Table 2.3.

Table 2.3: Uses of some *Goniothalamus* species in traditional medicine

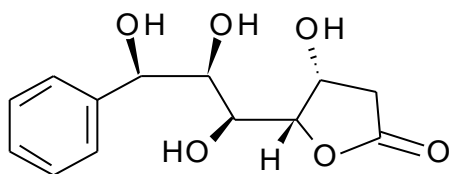
Species	Uses	References
<i>Goniothalamus curtisii</i>	Diarrhea	Mat Salleh and Latiff (2002)
<i>Goniothalamus giganteus</i>	Post-parturition	Wuart (2000)
<i>Goniothalamus macrophyllus</i>	Abortion, fever	Burkill (1953)
<i>Goniothalamus macrophyllus</i>	Abortifacient, fever, post-parturition	Wuart (2000)
<i>Goniothalamus malayanus</i>	Abortifacient, diarrhea, fever, headache, measles, post-parturition, rheumatism	Mat Salleh and Ahmad (1989); Latiff (1991)
<i>Goniothalamus scortechinii</i>	Abortion, insect bites, jaundice, post-partum protective remedy, post-parturition	Mat Salleh and Latiff (2002); Burkill (1953)
<i>Goniothalamus tapis</i>	Abortion, stomachache, diarrhea	Latiff (1991); Wuart (2000); Ahmad et al (2001); Burkill (1953)

2.3 Phytochemical Studies of *Goniothalamus* species

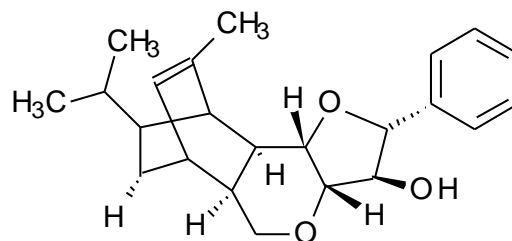
Goniothalamus species are rich in term of secondary metabolites and the major classes of chemical compounds isolated includes flavanoids (Surivet and Vatele, 1999), styryl-lactones (Colegate *et al.*, 1997), alkaloids (Cao *et al.*, 1997) and acetogenins (Cao *et al.*, 1997). Both categories of acetogenins and styryl-lactone posses complex stereochemistry. They appear in different stereoisomeric forms with multiple levels of cytotoxicity towards several human tumor cell lines. More than twenty styryl-lactones are known and most of them are stereoisomers (Hisham *et al.*, 2000).

2.3.1 Styryl-Lactones

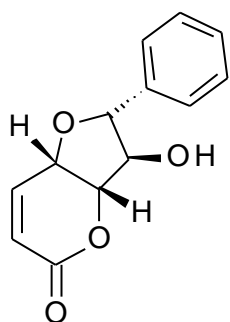
Styryl-lactones can be easily isolated from the *Goniothalamus* species. Styryl-lactones are low molecular weight phenolic compounds found in members of the Annonaceae family and present a lactonic pharmacophore (Wiar, 2006). Styryl-lactones are an interesting group of bioactive agents with significant cytotoxicities against several human tumor cell lines which many of the compounds in this group have been isolated from *Goniothalamus* species (Blazquez *et al.*, 1999). Cardiobutanolide (**1**), cardiopetalolactone (**2**), altholactone (**3**) and goniopyrone (**4**) were isolated from the stem bark of *Goniothalamus cardiopetalus* (Hisham *et al.*, 2000).



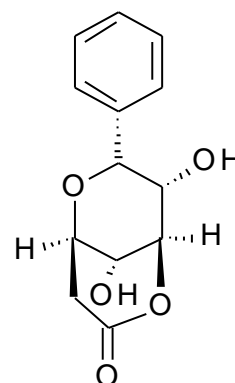
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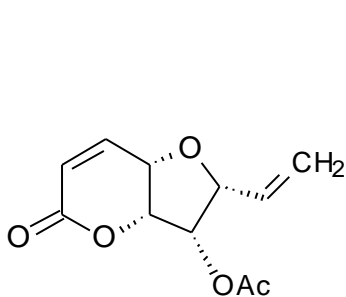
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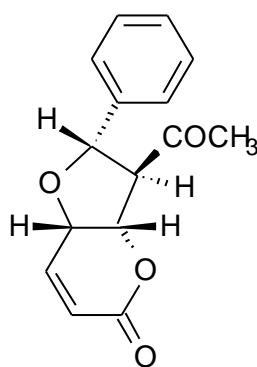
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Studies on *Goniothalamus arvensis* afforded the isolation of furano-pyrone (**5**), 3-acetylaltolactone (**6**) (Peris *et al.*, 2000), altholactone (**7**) (Loder and Nearn, 1997) and 5-acetoxyisogoniothalamine oxide (**8**) (Hasan *et al.*, 1994). Compounds **6**, **7** and **8** are cytotoxic based on the inhibition of mammalian mitochondrial respiratory chain (Peris *et al.*, 2000).

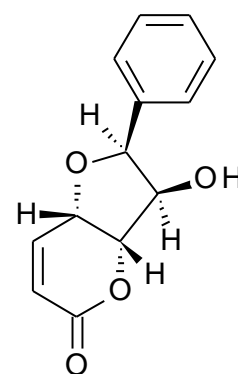
Studies on the stem bark of *Goniothalamus arvensis* afforded (+)-garvensintriol (**9**) and (+)-etharvendiol (**10**) together with (+)-goniofufurone (**11**) a known compound previously isolated from *Goniothalamus giganteus* (Bermejo *et al.*, 1997).



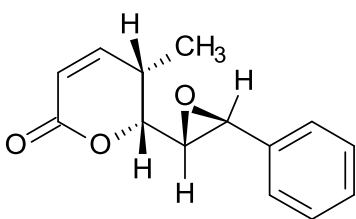
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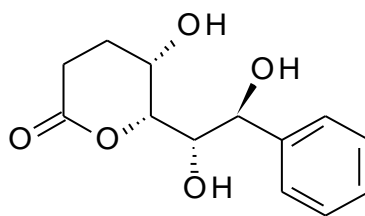
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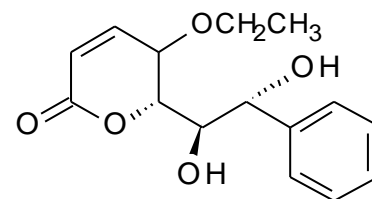
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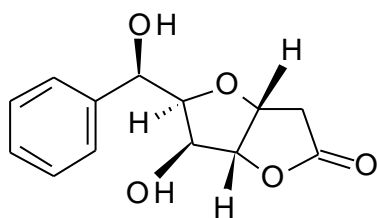


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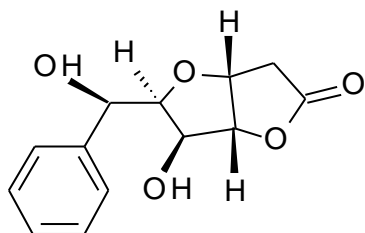
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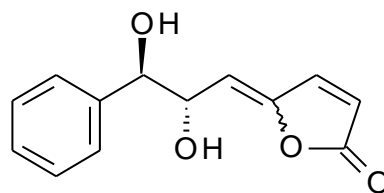


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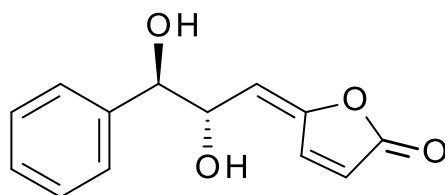
Various styryl-lactones have been isolated from the stem bark of *Goniothalamus giganteus* and some of the compounds include (+)-goniofufurone (**12**), goniobutenolides A (**13**), goniobutenolides B (**14**), (+)-gonioppyrone (**15**) and (+)-7-epi-goniofufurone (**16**) (Surivet and Vatele, 1999).



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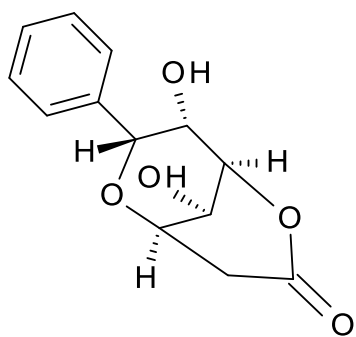


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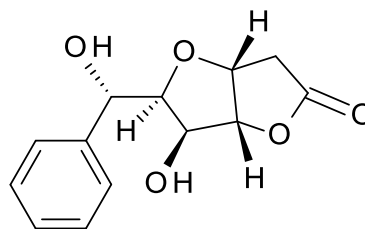


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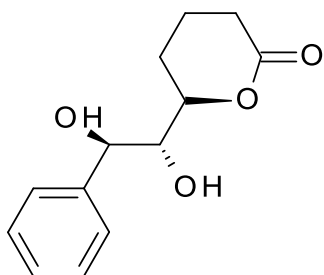


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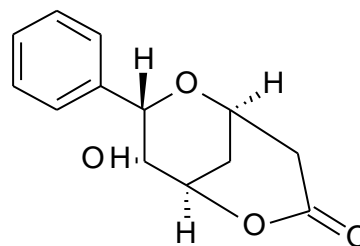


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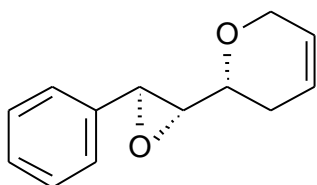
(+)-Goniodiol (**17**), (+)-9-deoxygoniopyrone (**18**), (-)-isogoniothalamine epoxide (**19**) and (-)-8-epi-9-deoxygoniopyrone (**20**) were isolated from *Goniothalamus dolichocharpus* (Surivet and Valette, 1999).



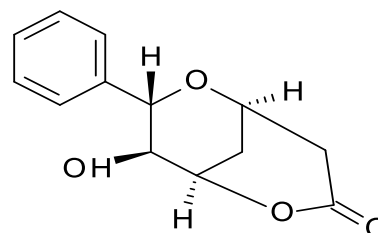
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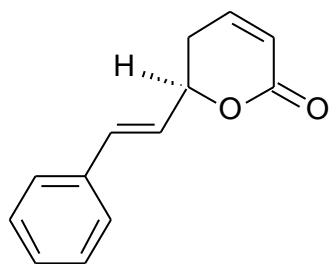


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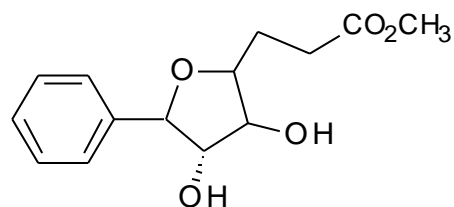


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Goniothalamins (**21**) are common styryl-lactones and have been isolated from various *Goniothalamus* spp. such as *Goniothalamus andersonii* (Hisham *et al.*, 2000). Goniothalesdiol (**22**) is another type of styryl-lactone that has been isolated from *Goniothalamus bornensis* (Cao *et al.*, 1997).



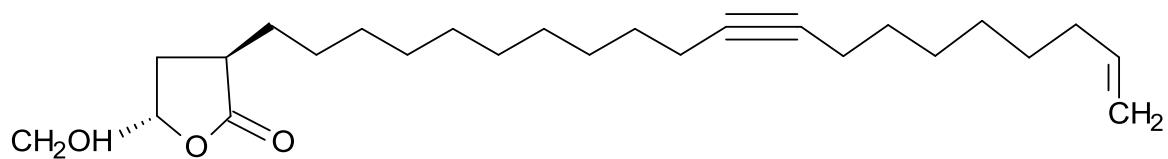
21



22

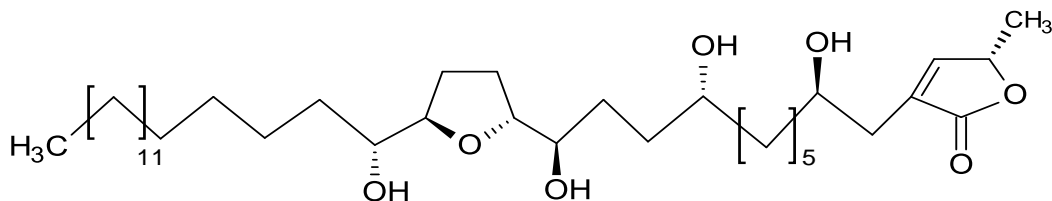
2.3.2 Acetogenins

This class of compound possesses a tetrahydrofuran (THF) ring with one or two hydroxyl groups and various terminal lactone rings (Atta-Ur-Rahman, 2006). Acetogenins also known as Annonaceae acetogenins is natural polyketides which have anticancer and pesticidal properties. Goniothalamusin (**23**) has been isolated from the petrol extract of the aerial parts of *Goniothalamus gardneri* (Seidel *et al.*, 1999). Goniothalamusin (**23**) is a linear acetylenic and olefinic acetogenin with a C₂₅ skeleton (Seidel *et al.*, 1999).

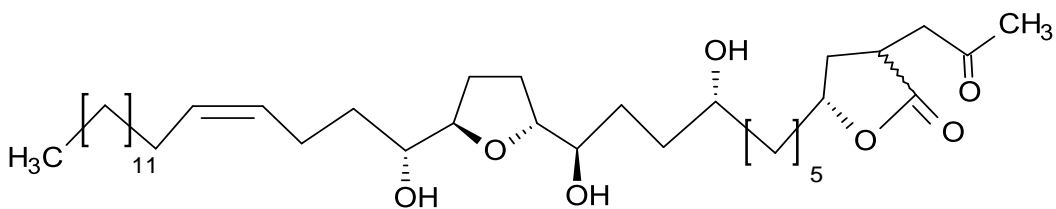


23

Another compound of this group that have been isolated are goniotetracin (**24**) and mixture of (2, 4-cis and trans)-gonioeninone (**25**) were isolated from the bark of *Goniothalamus giganteus* (Alali *et al.*, 1998).



24



25

Another two new acetogenins which are gardnerilins A (**26**) and gardnerilins B (**27**) have been isolated from the roots of *Goniothalamus gardneri* (Chen *et al.*, 1998).