PHYTOCHEMICAL STUDIES AND BIOLOGICAL ACTIVITIES OF THE METHANOL EXTRACT OF *PEPEROMIA PELLUCIDA*

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This project is submitted in partial fulfillment of the requirements for the degree of

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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 of institution of higher learning

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LIST OF ABBREVIATIONS

TLC	Thin Layer Chromatography
UV	Ultraviolet
GC-MS	Gas Chromatography- Mass Spectometry
DCM	Dichloromethane
EtOAc	Ethyl Acetate
MeOH	Methanol
µL	microlitre
mg	milligram
g	gram
cm	centimeter
mm	millimeter
h	hours

Phytochemical Studies and Biological Activities of The Methanol Extract of *Peperomia pellucida*

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ABSTRACT

The phytochemical and biological activities studies of methanol extracts of *Peperomia pellucida* is a study to determine the chemical components exist in the species, toxicity and the antibacterial activity of the crude extract. The yield of crude extract obtained was 6.32%. The crude was fractionated into number of fractions by using column chromatography. Fraction was further purified by using 20 cm x 20 cm Thin Layer Chromatography (TLC) plate to isolate the mixture of compounds. The separated band from TLC plate was analyzed by using Gas Chromatography- Mass Spectroscopy (GC-MS). Two major peaks were shown at retention time 21.921 minute and 23.147 minute. Ion fragmentation from MS data shown that compound at 21.923 min gave M^+ at m/z 286 and base peak at m/z 159.0. The compound at 23.147 min gave M^+ at m/z 259.2 and base peak at m/z 129.1. The crude extract was tested for cytotoxicity test on the larvae of *Artemia salina* and antibacterial test towards *Staphylococcus aureus* and *Escherichia coli*. Result data indicating that methanol crude extract shows growth inhibition for *E. coli* at 1% and 10% concentration, but no inhibition of growth shown for *S. aureus.* Toxicity test shows that the crude extract is not toxic at the concentration level tested which are 1 µg/mL, 10 µg/mL and 100 µg/mL.

Keywords: ***Peperomia pellucida,*** **antibacterial activity, cytotoxicity test,** ***Staphylococcus aureus, Escherichia coli*** **and** ***Artemia salina.***

ABSTRAK

Kajian fitokimia dan aktiviti biologi terhadap ekstrak metanol tumbuhan Peperomia pellucida merupakan kajian untuk menentukan komponen kimia yang hadir dalam spesis tumbuhan tersebut, ketoksikan dan aktiviti ekstrak kasar terhadap bakteria. Hasil ekstrak kasar yang diperoleh adalah sebanyak 6.32%. Ekstrak kasar difraksikan kepada beberapa pecahan dengan menggunakan kromatografi turus graviti. Pecahan yang terpilih ditulenkan dengan menggunakan Kromatografi Lapisan Nipis (KLN) bersaiz 20 cm x 20 cm untuk memisahkan campuran sebatian. Jalur yang dipisahkan daripada KLN dianalisis dengan menggunakan Kromatografi Gas-Spektroskopi Jisim (KG-SJ). Dua puncak utama ditunjukkan pada sela masa 21.921 minit dan 23.147 minit. Fragmentasi ion daripada data Spektroskopi Jisim menunjukkan sebatian pada minit ke 21.921 memberikan M^+ pada m/z 286 dan puncak asas pada m/z 159.0. Sebatian pada sela masa 23.147 min pula memberikan M^+ pada m/z 259.2 dan puncak asas pada m/z 129.1. Ekstrak kasar diuji dengan ujian ketoksikan terhadap larva Artemia salina dan ujian antibakteria dilakukan terhadap Staphylococcus aurues dan Escherichia coli. Keputusan menunjukkan bahawa ekstrak kasar tersebut menunjukkan kesan membantutkan pertumbuhan E. coli pada kepekatan 1% dan 10%, tetapi tidak menunjukkan kesan terhadap pertumbuhan S. aureus. Ujian ketoksikan pula menunjukkan ekstrak kasar tersebut tidak menunjukkan kesan toksik pada kepekatan yang diuji iaitu pada kepekatan 1 µg/mL, 10 µg/mL, dan 100 µg/mL.

Kata kunci : Peperomia pellucida, aktiviti antibakteria, ujian ketoksikan, Staphylococcus aureus Escherichia coli dan Artemia salina.

CHAPTER 1

INTRODUCTION

1.1 General Introduction

The genus *Peperomia* belongs to the family of Piperaceae which consist of three other major genera including *Piper, Sarchorhanchis* and *Ottonia*. Piperaceae has considerable economic importance, as most of its members used as ornamentals, apart from their potential in medicinal and culinary area. This type of plant can be found within tropical and subtropical region (Kato and Furlan, 2007). *Piper* and *Peperomia* represent 2000 and 1700 species (Felippe *et al.*, 2008) respectively.

Peperomia species are known as epiphytes or succulent plants live in moist forest and highland areas (Kato and Furlan, 2007). Species of *Peperomia* are well known as ornamental plants. Yet, some *Peperomia* species have been used as folk medicine. For example, *Peperomia vulcanica* Baker & C.H Wright is being used against sterility and *Peperomia japonica* Makino is used for the treatment of malignant tumors (Mbah *et al.*, 2002). *Peperomia* has been applied in folk medicine for the treatment of inflammation, asthma and gastric ulcers and as analgesic and antibacterial agent (Felippe *et al.*, 2008).

In this study, *Peperomia pellucida* has been used. *Peperomia pellucida* is a herbaceous plant that can be found easily at almost all over the world especially in the tropical region. According to Arrigoni-Blank *et al.* (2002) ,this plant is an annual herb and popularly known in northeast Brazil as “coracaozinho: (little heart), “lingua de sapo” (toad’s togue), “erva-de-vidro” (glass grass) and “erva-de-jabuti” (purpoise grass). In Malaysia, this plant is generally known as Kelampungan Air while the local Malay in Sarawak called this plant as “daun linyok” (greasy leaf). All the names given are due to the characteristic of the *P. pellucida* leaves which are shiny, greasy and wet-like. *P. pellucida* have alternate oval leaves and inflorescence, which grows well in loose and humid soil under the shades of tree (Arrigoni-

Blank, 2004). In folk medicine, this species is used to treat abscesses, boils and skin wounds, and eye inflammation (conjunctivitis) (Khan and Omoloso, 2002). This species also used as an emollient, diuretic and to control cough and cardiac arrhythmia (Bayma *et al.*, 2000).

From previous studies, this species has shown several biological activities such as analgesic activity in mice (Khan and Omoloso, 2002), anti-inflammatory effect (Arrigoni-Blank *et al.*, 2003) and also antipyretic effect (Khan *et al.*, 2007). Chemical studies on this plant established the occurrence of alkaloids, flavonoids, saponins, sterols, tannins and terpenoids (Khan, 2002). Due to the medicinal potential shown by this plant, further studies should be done to determine biological active compounds exist in this plant.

This research which involves the phytochemical and biological study of the methanol extract of *P. pellucida* is important to know the compounds exist in the crude extract together with their and cytotoxicity, and biological activity towards selected bacteria. Since ancient time, human get used to herbal plants as medicine to cure several types of diseases. Even until now, the use of herbal medicine is still applicable and yet become wider than before. Thus, further study should be done widely to determine the active compound exist in those herbal. This kind of research can contribute to the medicinal area as biologically active compound found can be used to cure various types of plant, animal and human diseases.

1.2 Objectives

The objectives of this study are:

- to extract, isolate and characterize compounds from *Peperomia pellucida.*
- to determine the antibacterial activity and cytotoxicity of the methanol extracts of *Peperomia pellucida* .

CHAPTER 2

LITERATURE REVIEW

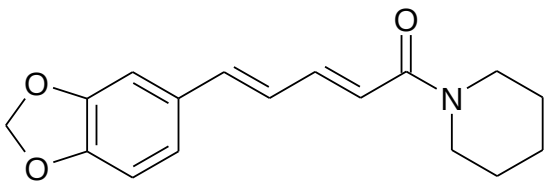
2.1 Piperaceae

Piperaceae distributed largely in tropical and subtropical regions of the world. *Piper* and *Peperomia* represent 2000 and 1700 species respectively (Felippe *et al.*, 2008).

2.2 Secondary metabolites from Piperaceae

The Piperaceae family showed the occurrence of phenylpropanoids, lignan/neolignans, pyrones, aliphatic and aromatic amides, alkaloids, polyketides and chromenes (Felippe *et al.*, 2008). Most of the studies reported to date concerned species of the genus *Piper* although species of the genus *Peperomia* can be found worldwide as they are used particularly in collections of ornamentals (Salazar *et al.*, 2005).

Chemical studies shown that the genus *Piper* has many components which included amides, flavonoids, lignans, aristolactams, long and short chain esters, terpenes, steroids, propenylphenols and alkaloid (de Morais *et. al.*, 2007). Piperine **(1)** was isolated form *Piper nigrum* (Prasad *et al.*, 2005).



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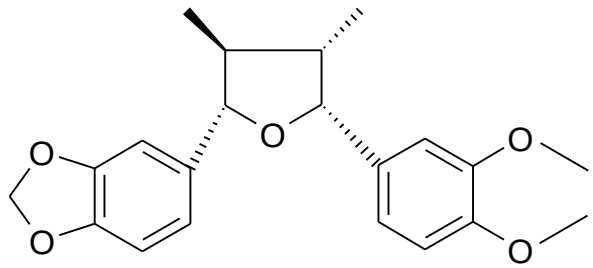
Lignan/neolignan compounds can be found in Piperaceae. For example, Calopiptin **(2)** from the group of 2,5-bisaryl-3,4-dimethyltetrahydrofurans was isolated from *Piper wightii*. Asarinin **(3)** was found in *Piper longum*, Piperone **(4)** in *Piper schmidtii*, and Lancifolin D **(5)** from the group 1,2-diarylpropanoids in *Piper argyrophyllum* (Prasad *et al.*, 2005).



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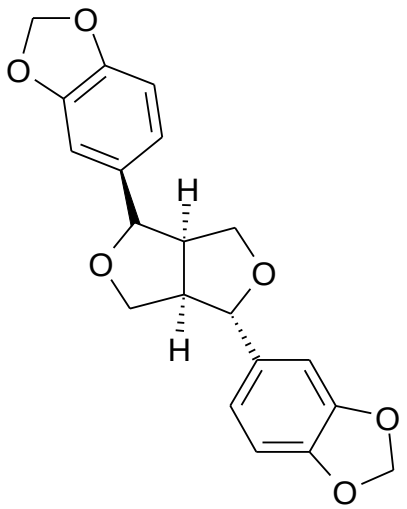

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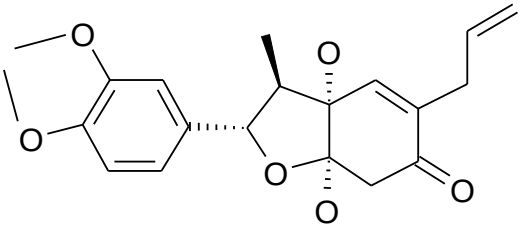

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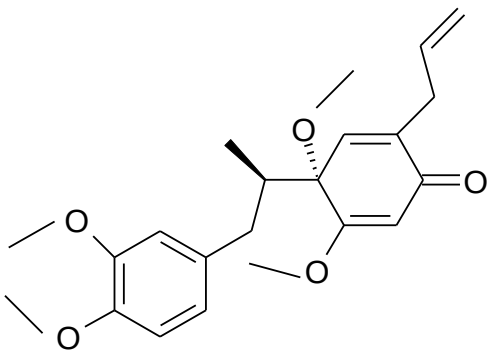

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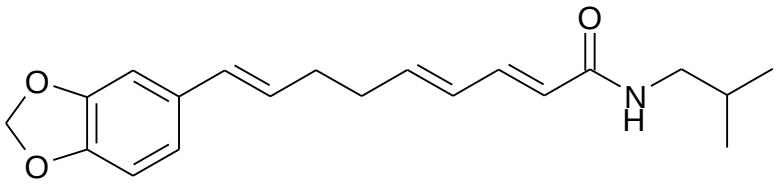
Retrofractamide A **(6)** is the example of amide that can be found in *Piper manii* and *Piper longum* (Prasad *et. al.*, 2005).



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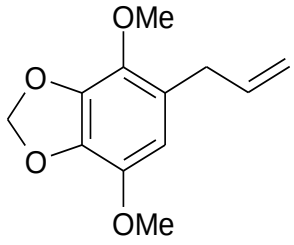
A tetrahydrofuran lignan and the known tetrahydrofuran lignan (-)-grandisin and five phenylpropanoid derivatives were isolated from *Piper solmsianum* (Martins *et al.*, 2000). The compounds are, Apiol (**7),** Dillapiole (**8),** Myristicin, (**9),** Elemicin (**10),** Isoelemicin (**11),** Grandisin (**12),** and Lignan (**13).**



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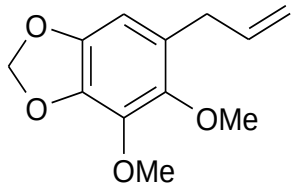

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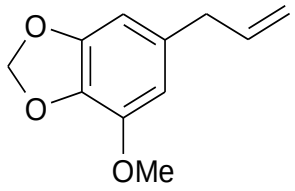

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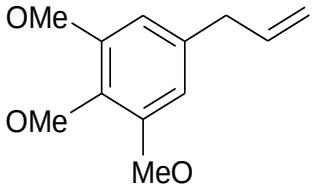

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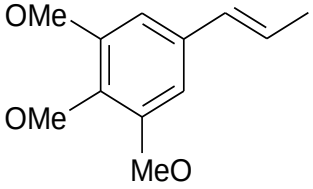

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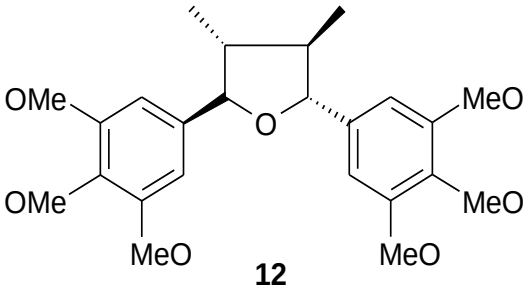

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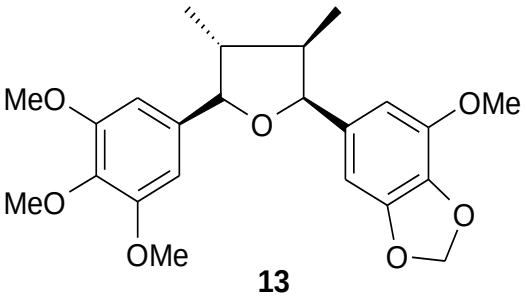

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2.3 Biological activities of Piperaceae

Extracts from various species of Piperaceae has been tested using antifungal bioassay and extract from *Piper hispidum* proved to be active against *Cladosporium sphaerospermum* and *Cladosporium cladosporioides* (Kato and Furlan *et al.*, 2007). Besides, the genus *Piper* has been an important source of secondary metabolites which have insecticidal activity such as several isobutylamides characterized as guineesine, pipercide and retrofractamide A isolated from *Piper nigrum* fruit showed activity against *Aedes aegypti*. (de Morais *et al.*, 2007). Ethanolic extracts derived from *Piper longum* L., *Piper ribesoides* Wall., and *Piper sarmentosum* Roxb. Ex Hunt. have efficiency against early fourth-instar larvae of *A. aegypti* mosquitoes using larvacidal bioassays (de Morais *et al.*, 2007).

2.4 *Peperomia* species

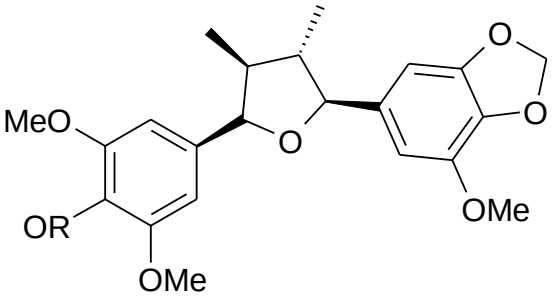
Peperomia species are quite commonly encountered as epiphytes or as succulent plants (Kato and Furlan, 2007). The genus *Peperomia* has over 1000 species occurring in the tropics, with variety size and shape of leaves, adapted to moist habitat and dry highlands as well (Lago *et al.*, 2007). Species of *Peperomia* are usually grown as a potted ornamental foliage plant. Species of *Peperomia* have been used in folk medicine for treatment of inflammation, asthma and gastric ulcers (Felippe *et al.*, 2007).

2.5 Secondary metabolites from *Peperomia species*

In comparison with the genus *Piper,* knowledge on the *Peperomia* is rather limited. Compounds derived from shikimate pathway includes secolignans, peperomins and styrene dimers present in *Peperomia* species (Kato and Furlan, 2007).

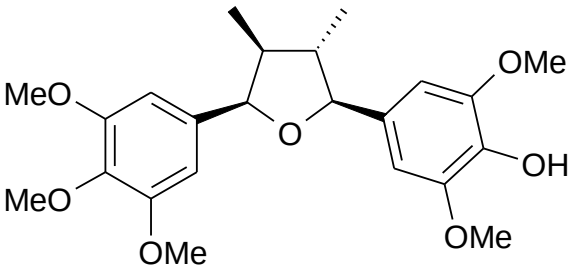
From the secondary metabolites obtained from *Peperomia* species, the most concerned are seco-compounds for example, the secolignans from *Peperomia glabella* (Salazar *et al.*, 2006). A number of acylphloroglucinol or phenolic compounds with long aliphatic chains have been isolated from *Peperomia vulcanica*, *Peperomia obtusifol*, *Peperomia galides,* and *Peperomia proctorii* (Salazar *et al.*, 2006).

Five tetrahydrofuran lignans **(14-18)** and two unknown flavones were isolated from the aerial parts of *Peperomia blanda* (Felippe *et al*, 2008).



14 R=H

15 R= Me



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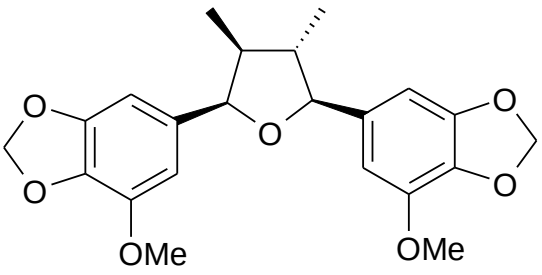

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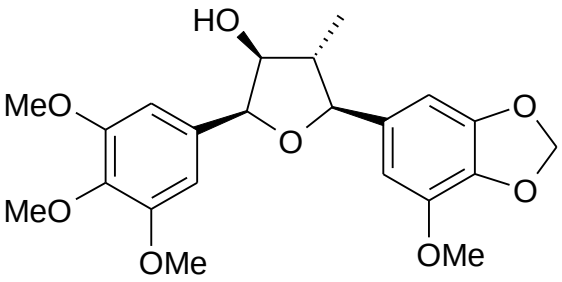

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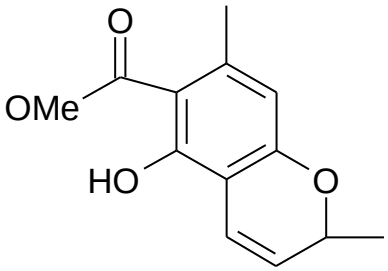
Peperomia villipetiola has been found to contain myristicin and seven chromenes **(19-25)** (Salazar *et al.,* 2005) as shown in **Figure 2.5.**



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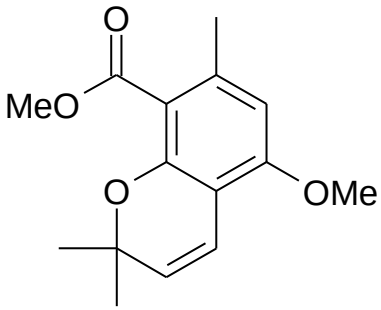

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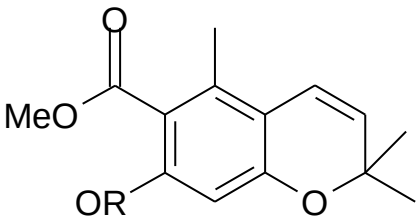
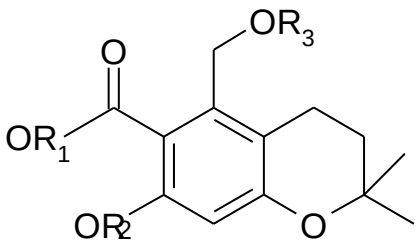


21 R= H

22 R= Me

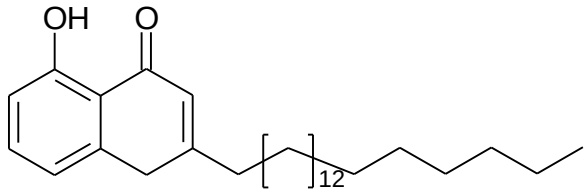


23 R_1=H ; R_2= H ; R_3= H

24 R_1= H ; R_2= Me ; R_3= H

25 R_1= Me ; R_2= H ; R_3= Ac

Two chromones ; 5-hydroxy-2-(14'-(E)-nonadecenyl) chromone **(26)** and 5-hydroxy-2-[12'-(3",4"-methylenedioxyphenyl)dodecanyl] chromone **(27)** was isolated from *Peperomia vulcanica* (Mbah *et al.,* 2002)



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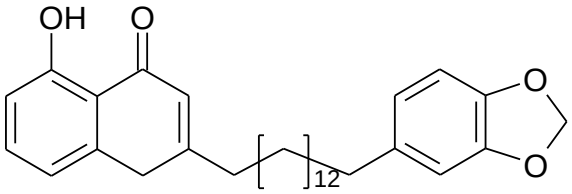

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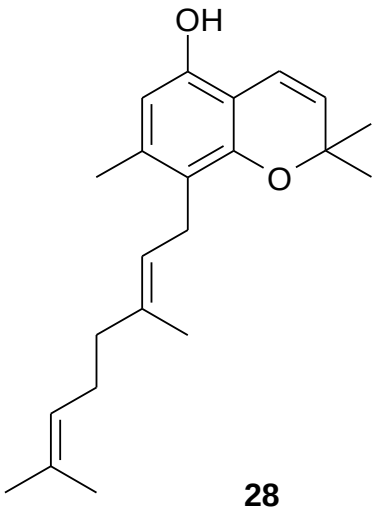
Chromatographic separation of the CH_2Cl_2 extract from leaves of *Peperomia serpens* yielded two chromenes [5-hydroxy-8-(3',7'-dimethylocta-2',6'-dienyl)-2,2,7-trimethyl-2H-1-chromene **(28)** and 5-hydroxy-8-(3'-methyl-2'-butenyl)-2,2,7-trimethyl-2H-1-chromene-6-carboxylic acid **(29)** besides the known chromene methyl 5-hydroxy-2,2,7-trimethyl-2H-1-chromene-6-carboxylate and the flavonoid, dihydrooroxylin (Kitamura *et al.,* 2006).



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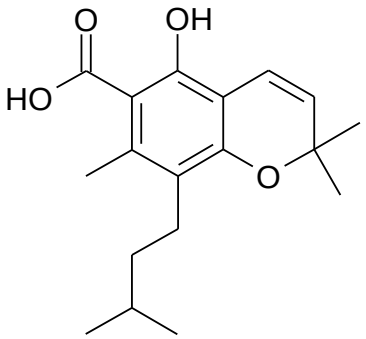

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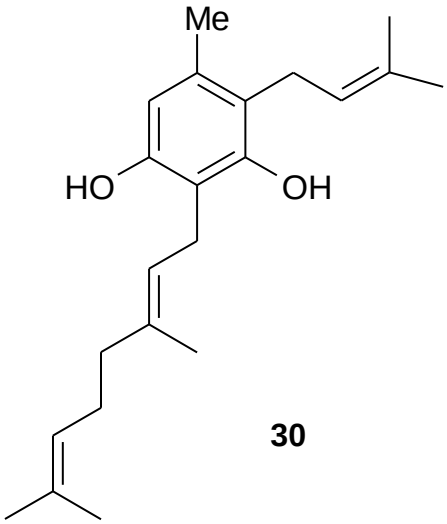
From the aerial parts of *Peperomia obtusifolia*, five phenolic compounds bearing a methyl, an isoprenyl and a geranyl group on a benzene ring core **(30-34)** has been isolated (Tanaka *et. al.*, 1997).



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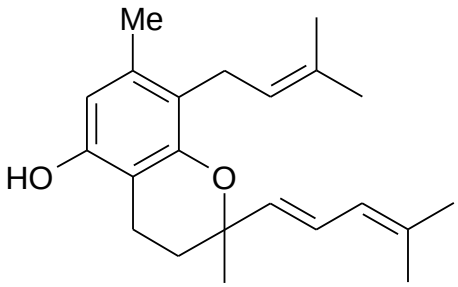

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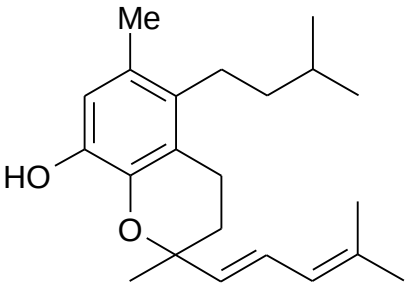

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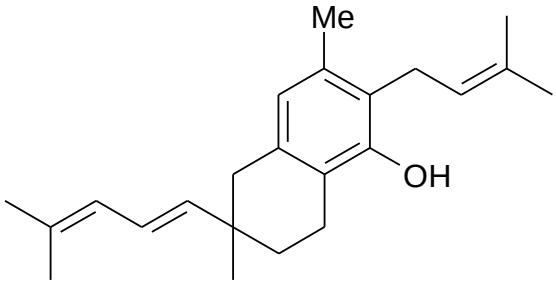

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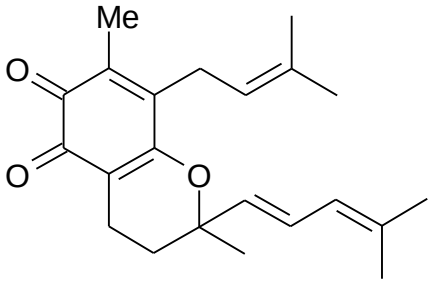

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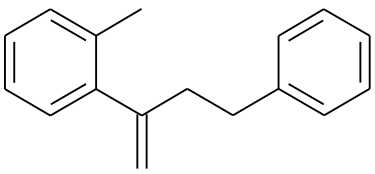

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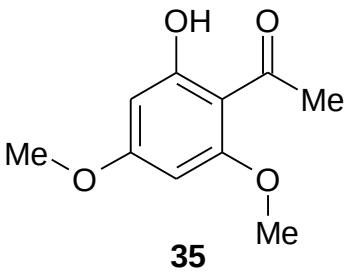
The 2-hydroxy-4,6-dimethoxyacetophenone **(35)** was isolated from the leaves of the *Peperomia glabella* (Soares *et al.*, 2006).



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2.6 Biological activities of *Peperomia species*

Peperomia villipetiola showed antifungal activity against *Cladosporium cladosporioides* (Salazar *et al.*, 2005). Isolation of antitumor dibenzylbutyrolactone derivative (Kato and Furlan, 2007) also had been done from *Peperomia* species. The crude extracts of *Peperomia serpens* showed higher activity towards *Cladosporium cladosporioides and C. sphaerospermum* rather than the pure compounds (Kitamura *et al.*, 2006).

Acetophenone derivatives from the leaves extract of *Peperomia glabella* shown many interesting biological properties such as anti-inflammatory, cytotoxic and choleretic activity (Kitamura *et al.*, 2006).