CHAPTER I

INTRODUCTION

1.1 Thai natural products as leads for drug candidates

Natural products still play a crucial role for drug discovery and development. They often provide lead compounds or structures which can further be developed to be a real drug [1, 2]. In some Asian countries, particularly India, China and Thailand, up to 50% of the population still relies on traditional medicines for their primary health care needs. Medicinal herbs used as folk remedies have attracted considerable attention from natural product and medicinal scientists as sources of targeted active substances, since they have been exploited for the treatment of human diseases such a long time ago. In addition, understanding of therapeutic benefits deepens and demands for natural products increase, previously accidental discoveries thus evolve into active searches for new medicines.

To look for drug candidates, it is important to realize the methods and rationale utilized to provide the best opportunities to obtain the natural sources that can produce the interesting metabolites. One is the screening of extracts of those sources for the presence of new compounds and an investigation of their targeted activity. Interestingly, selection of plants or natural sources is also a principle role, and one of them is plants growing in areas of great biodiversity, for example, in tropical area. Therefore Thai medicinal plants should be a great home of drug leads, since Thailand is located near the equator and the climate is generally hot and humid. Experiences and successes of Thai scientists in this specialized area have resulted in a number of widely commercial drugs, although not in single-drug form; however, their active principles have been identified. The most popular ones are *Andrographis paniculata* and *Curcuma longa* capsules (Figure 1.1) for curing fever and indigestion, respectively.

(a) (b)

Figure 1.1 Thai medicinal plant drugs: (a) *Andrographis paniculata* capsules (b) *Curcuma longa* capsules (Image from: http://www.gpoproduct.com/tabid/39/language/th-TH/Default.aspx) (Site on November 25, 2012)

1.2 Plants in the genus *Harrisonia*

Among the medicinal plants available in Thailand, plant in the genus *Harrisonia* (family Simaroubaceae) is one of the most widely used herbs in traditional Thai medicines. The genus *Harrisonia* comprises three species including *H. perforata*, *H. brownii* and *H. abyssinica* (Oliv.). However, *H. perforata* (Blanco) Merr. or Khonthaa in Thai is the only species of this genus growing in Thailand, and is applied in Thai folklore medicine. Its dried root is considered antipyretic and anti-inflammatory, and it is utilized as a remedy for the treatment of wound healing and diarrhea [3].

1.2.1 Botanical characteristics of *Harrisonia perforata*

H. perforata belongs to the family Simaroubaceae. The taxonomic classification of this plant is shown in Table 1.1.

Table 1.1 Taxonomy of *Harrisonia perforata*

Kingdom	Plantae
Class	Rosopsida
Order	Sapindales
Family	Simaroubaceae
Genus	Harrisonia
Species	H. perforata

H. perforata is a shrub or small tree native to Southeast Asia including Thailand. Its habitat is usually in dry, open localities such as light secondary forest, thickets and forest edges, often on limestone rocks, less common in monsoon forest. It prefers distinctly seasonal conditionals from sea-level up to 700-900 m altitude. *H. perforata* is a scandent to erect prickly shrub up to 4-6 m tall. The leaves pinnate with unpaired terminal leaflet up to 20 cm long, with 1-15 pairs of leaflets supported by a 5-30 mm long stalk. The stipulate thorns are slightly curved backward or downward, increasing in size to 7 mm. Its leaflets are rhomboid to ovate-lance-shaped, 10-20 mm × 5-15 mm, nearly entire to lobed with narrowly winged rachis. Flowers are with a pedicel, small sepal, triangular lobes, petals are lance-shaped, 6-9 mm × 2-4 mm which are red outside and pale red to white inside. The stamens are (8-)10 with anthers 1.5-4.5 mm long, filaments are 7-10 mm long, at the base with an elongated flattened strap-shaped structure which is densely woolly at the margin, disk is cup-shaped, ovary is slightly lobed, styles 5-8 mm long and pubescent. The fruit is a berry, 4-9 mm × 11-15 mm, exocarp of leathery texture, at least 1 mm thick, endocarp hard, without suture. The picture of the parts of *H. perforata* is depicted in Figure 1.2.

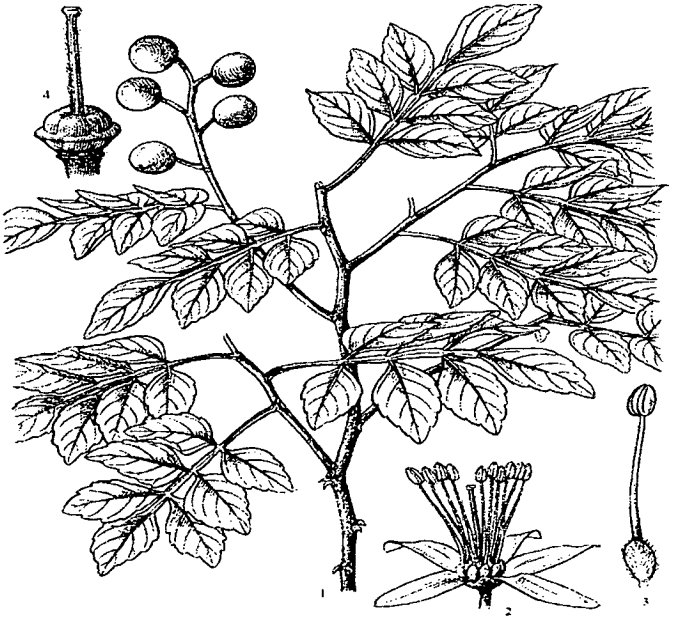
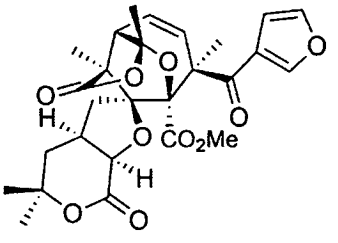


Figure 1.2 Parts of *Harrisonia perforata*: 1) fruiting branch, 2) flower, 3) stamen with scales at base of filament, 4) pistil
(Image from: http://www.efloras.org/object_page.aspx?object_id=109692&flora_id=2) (Site on January 5,2013)

1.2.2 Secondary metabolites of the genus *Harrisonia*

Chemical constituents mainly found in plants in the genus *Harrisonia* were chromones and highly rearranged limonoids. A number of examples are presented here.

In 1994 Ohmoto and co-workers reported the isolation and characterization of two new rearranged limonoids, namely brownins C (**1**) and G (**2**) from the bark and the wood of *H. brownii*, respectively [4].



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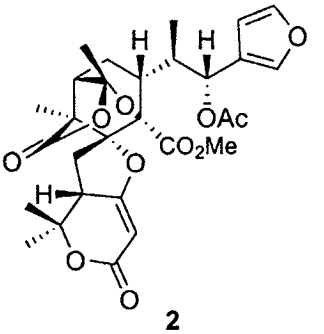
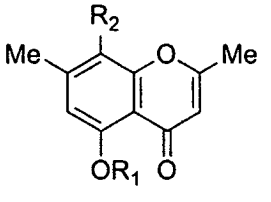
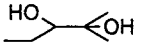
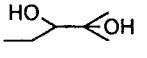
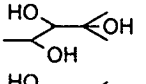
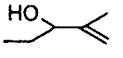
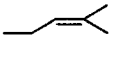
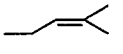
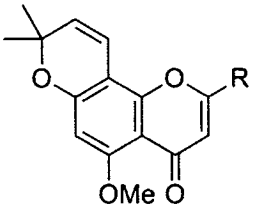


2

In 1995 Koike and co-workers described the isolation and structural elucidation of five new chromones, perforatins C-G (**3-7**), along with six known derivatives (**8-13**), from the wood of *H. perforata* collected in Hainan, China [5].

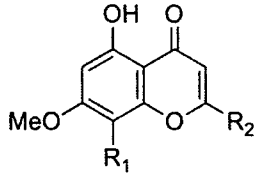


	R_1	R_2
3	H	
4	Me	
5	Me	
6	Me	
8	H	
9	Me	

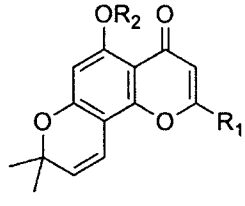


	R
7	CH_2O-β-D-glc
10	CH_2OH
11	Me
12	COOH
13	COOMe

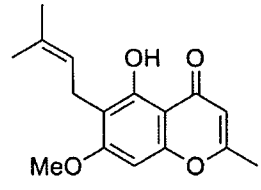
In addition, a number of chromones from the branches of *H. perforata* collected in Thailand were reported in the next year by Tuntiwachwuttikul and co-workers [6]. Four new chromones namely perforamones A-D (**14-17**), together with six known analogs (**18-23**), were isolated and identified. All isolated compounds were tested for their antiplasmodial and antimycobacterial activities. Only compound **21** showed good activity on antiplasmodial assay with an EC_{50} value of 10.5 μg/mL, while most of them exhibited antimycobacterial activity with MIC values ranging from 25-200 μg/mL.



	R_1	R_2
14		Me
15		Me
16		Me
18	H	Me
19	H	CH_2OH



	R_1	R_2
17	H	CH_2OH
20	COOH	Me
21	Me	Me



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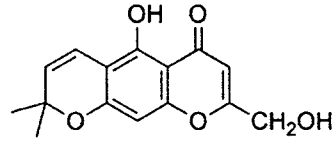
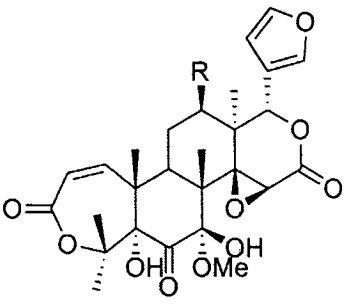


23

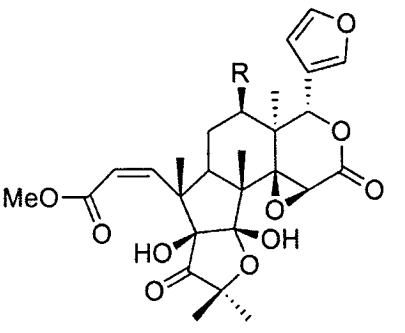
In 1997 Fischer and co-workers have revised the structures of two known limonoids, harrisonin (**24b**) and 12β-acetoxyharrisonin (**25b**), from the root of *H. abyssinica*, which have been previously reported as **24a** and **24b**, respectively [7]. Structure revision of these limonoids was peformed by reanalysis with modern 1D- and 2D-NMR spectroscopic methods and, in addition, by single crystal X-ray diffraction for the latter one [8].



R

24a H

25a OAc

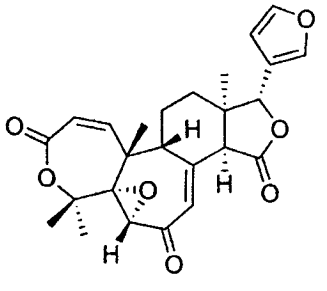


R

24b H

25b OAc

In 2001 Khuong-Huu and co-workers described the isolation and identification of three new rearranged limonoids, haperforins C2 (**26**), F (**27**) and G (**28**), from *H. perforata* leaves collected in Central Vietnam. Their structures were mainly determined by analysis of single-crystal X-ray diffraction data [9].



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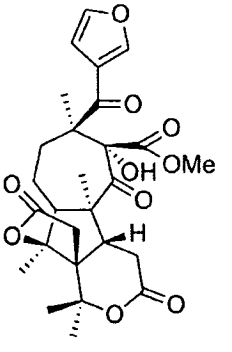

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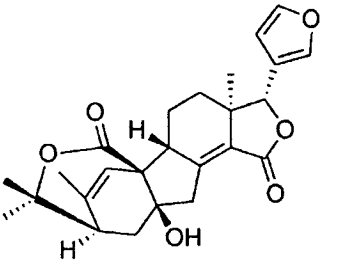

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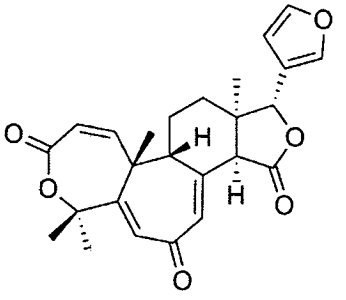
In 2011 chemical constituents of fruits of *H. perforata* was first studied by Hao and co-workers. Plant samples were collected from Hainan, China. This study has led to the isolation and characterization of three new limonoids with highly rearranged A, B and D-*seco*-16-nor skeletons (**29-31**). Isolated limonoids were tested for their cytotoxicity toward human leukaemia (HL-60) and lung adenocarcinoma (A-549) cell lines. Only limonoid **30** exhibited weak activity with inhibition rates of 63.6 and 64.9%, respectively, whereas the other compounds were shown to be inactive [10].



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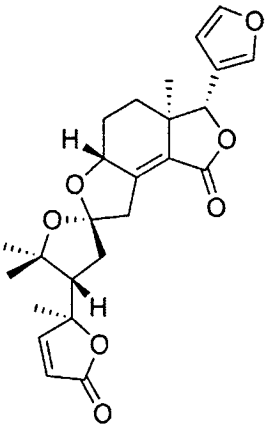

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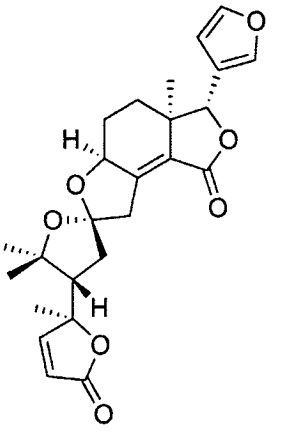

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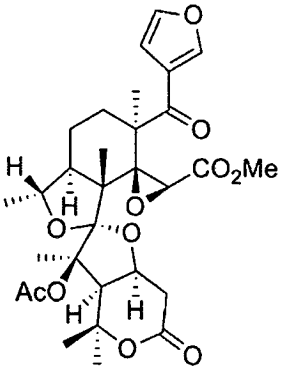

32

In the year 2000, Païs and co-worker have also reported the isolation and identification of a new rearranged limoniod with D-ring cleavage, foritin (**32**), from *H. perforata* bark, collected in Hanoi, Vietnam [11].

1.3 Aim and scope of the present study

As presented above, secondary metabolites isolated from various parts of *H. perforata*, mostly collected in China and Southeast Asia, have considerably attracted attention from natural product researchers, including our group. This is because of the unique structures of rearranged limonoids found in this plant. Study of *H. perforata* chemical constituents collected in Thailand, was performed only by one research group, Tuntiwachwuttikul and co-workers, and only one report has been internationally published; however, only chromone derivatives, no any limonoids, have been isolated [6]. Most importantly, the anti-inflammatory activity of its

metabolites has not been studied yet, although *H. perforata* is utilized in traditional medicine for wound healing treatment and considered anti-inflammatory. In the present study, based on its application in folklore medicine, the isolation and characterization of the constituents of *H. perforata* found in Thailand would thus be performed, and anti-inflammatory agents would further be identified.

The objectives of this research could be divided into the following three parts as follows:

1. To extract, isolate and purify the chemical constituents of *H. perforata* fruits and roots collected in Sukhothai and Nakornsawan province, respectively.

2. To elucidate the structures of the isolated compounds from *H. perforata* fruits and roots by spectroscopic techniques.

3. To evaluate the anti-inflammatory activity of the isolated metabolites using nitric oxide inhibitory assay.