Chemical constituents and nitric oxide inhibitory activity of capparis micracantha and maerua siamensis

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CHEMICAL CONSTITUENTS AND NITRIC OXIDE INHIBITORY ACTIVITY OF CAPPARIS
MICRACANTHA AND MAERUA SIAMENSIS

Miss Sasiwimon Nukulkit

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Pharmacognosy
Department of Pharmacognosy and Pharmaceutical Botany
FACULTY OF PHARMACEUTICAL SCIENCES
Chulalongkorn University
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องค์ประกอบทางเคมีและฤทธิ์ยับยั้งไนตริกออกไซด์ของชิงชี่และแจง

น.ส.ศศิวิมล นุกูลกิจ
วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต สาขาวิชานำสัตว์ ภาควิชาเภสัชเวทและเภสัชพฤกษศาสตร์ คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ปีการศึกษา 2565 ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย
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ศศิวิมล นุกูลกิจ : องค์ประกอบทางเคมีและฤทธิ์ยับยั้งไนตริกออกไซด์ของชิงชี่และแจง. (CHEMICAL CONSTITUENTS AND NITRIC OXIDE INHIBITORY ACTIVITY OF CAPPARIS MICRACANTHA AND MAERUA SIAMENSIS) ที่ปรึกษาหลัก : ผศ. ดร.ชัยศักดิ์จันศรีนิยม ที่ปรึกษาร่วม : รศ. ดร.รุทธิสุทธิศรี,ดร.มัตถกาคงขาว

การศึกษาองคประกอบทางเคมีของพืช 2 ชนิดในวงศ์ Capparaceae คือชิงชี่และแจงสามารถสกัดแยกสารบริสุทธิ์ได้ทั้งหมด 13 ชนิด จากการพิสูจน์โครงสร้างด้วยเทคนิคทางสเปกโทโลโคปี รวมกับการเปรียบเทียบกับสารที่เคยมีการรายงานมาก่อน พบสารที่มีรายงานแล้วทั้งหมด 5 ชนิด จากส่วนลำต้นของชิงชี่ (Capparis micracantha) ประกอบด้วยสารในกลุ่มอินโดอลคาลลอยด์ 1 ชนิด คือ methyl 6-methoxy-3-indolecarbonate, สารในกลุ่มอนุพันธ์ของกรดเบนโซอิก 1 ชนิด คือ vanillic acid, สารในกลุ่มเดิมก้าน 1 ชนิด คือ (−)-syringaresinol และสารในกลุ่มสติลบีนไดเมอร์ 2 ชนิด คือ (+)-ampelopsin A และ (−)-pauciflorol E สำหรับรากของแจง (Maerua siamensis) พบสารใหม่ทั้งหมด 8 ชนิด ซึ่งเป็นสารในกลุ่มอินโดอลคาลลอยด์ ได้แก่ (+)-maeruanitrile A, maeruanitrile B, maeroximes A - C และ maeruabisindoles A - C สารที่แยกได้ทั้งหมดถูกนำมาทดสอบฤทธิ์ยับยั้งการสร้างไนตริกออกไซด์ ในเซลล์แมโครฟาจ RAW 264.7 ที่ถูกเหนี่ยวนำด้วยลิโปพอลิแซ็กคาไรด์เปรียบเทียบกับสารควบคุมผลบวก indomethacin พบว่าสาร (−)-pauciflorol E และ methyl 6-methoxy-3-indolecarbonate จากลำต้นชิงชี่ มีค่าความเข้มข้นที่ยับยั้งการสร้างไนตริกออกไซด์ได้ 50 เปรอร์เซ็นต์ IC₅₀ เพื่อพบ 123.40 ± 4.51 และ 198.00 ± 5.57 ไมโครโมลาร์ ตามลำดับ สำหรับสาร maeruabisindoles B-C, maeroxime C, (+)-maeruanitrile A และ maeruanitrile B จากรากแจง มีค่า IC₅₀ เพื่อพบ 31.1 ± 1.04, 56.7 ± 2.2, 92.2 ± 5.1, 186.4 ± 13.0 และ 186.8 ± 13.3 ไมโครโมลาร์ ตามลำดับ ในขณะที่สาร indomethacin มีค่า IC₅₀ อยู่ในช่วง 150.0 - 166.3 ไมโครโมลาร์ การศึกษานี้สนับสนุนการใช้ลำต้นชิงชี่และรากของแจงเพื่อต้านอักเสบตามภูมิปัญญาการแพทย์แผนไทย
ABSTRACT (ENGLISH)

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Sasiwimon Nukulkit : CHEMICAL CONSTITUENTS AND NITRIC OXIDE INHIBITORY ACTIVITY OF CAPPARIS MICRACANTHA AND MAERUA SIAMENSIS.

Advisor: Asst. Prof. CHAI SA K CHANSRIN YOM, Ph.D. Co-advisor: Assoc. Prof. RUTT SUTTISRI, Ph.D., Mattaka Khongkow, Ph.D.

The chemical investigation of two plants in Capparaceae family, which are Capparis micracantha and Maerua siamensis, leads to the isolation of 13 compounds. The structures of the isolates were elucidated using spectroscopy techniques and comparison of the previous reports. Of five known compounds from the stems of C. micracantha were an indole alkaloid (methyl 6-methoxy-3-indolecarbonate), a benzoic acid derivative (vanillic acid), a lignan [(−)-syringaresinol], and two stilbene dimers [(+)-ampelopsin A and (−)-pauciflorol E]. In addition, eight new indole alkaloids named (+)-maeruanitrile A, maeruanitrile B, maeroximes A-C and maeruabisindoles A-C were isolated from the roots of M. siamensis. All isolates were tested for inhibition of nitric oxide production in lipopolysaccharide-induced RAW 264.7 macrophage cells compared with a positive control (indomethacin). (−)-Pauciflorol E and methyl 6-methoxy-3-indolecarbonate from C. micracantha stems exhibited half maximum inhibitory concentration (IC₅₀) values of 123.40 ± 4.51 and 198.00 ± 5.57 µM, respectively. Moreover, maeruabisindoles B - C, maeroxime C, (+)-maeruanitrile A and maeruanitrile B from M. siamensis roots showed IC₅₀ values of 31.1±1.04, 56.7 ± 2.2, 92.2 ± 5.1, 186.4 ± 13.0 and 186.8 ± 13.3 µM, respectively, while the IC₅₀ of indomethacin was in the range of 150.0 - 166.3 µM. This study supports the use of C. micracantha stems and M. siamensis roots for anti-inflammatory according to Thai traditional medicine knowledge.
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Sasiwimon Nukulkit
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LIST OF ABBREVIATIONS

acetone-$d_6$ = Deuterated acetone
$br~s$ = Broad singlet (for NMR spectra)
$^\circ C$ = Degree Celsius
CC = Column Chromatography
CDCl$_3$ = Deuterated chloroform
CH$_2$Cl$_2$ = Dichloromethane
cm = Centimeter
cm$^{-1}$ = reciprocal centimeter (unit of wave number)
$^{13}$C-NMR = Carbon-13 Nuclear Magnetic Resonance
1D NMR = One-dimensional Nuclear Magnetic Resonance
2D NMR = Two-dimensional Nuclear Magnetic Resonance
d = Doublet (for NMR spectra)
$dd$ = Doublet of doublets (for NMR spectra)
DMSO = Dimethylsulfoxide
$\delta$ = Chemical shift (in part per million unit)
DEPT = Distortionless Enhancement by Polarization Transfer
$\epsilon$ = Molar absorptivity
ESI-MS = Electrospray Ionization Mass Spectrometry
EtOAc = Ethyl acetate
et al. = et alibi (and other)
FCC = Flash Column Chromatography
$g$ = Gram
HMBC = Heteronuclear Multiple Bond Correlation
HR = High Resolution
$^1$H-NMR = Proton Nuclear Magnetic Resonance
HSQC = Heteronuclear Single Quantum Coherence
Hz = Hertz
IC$_{50}$ = Half maximal inhibitory concentration
IR = Infrared spectrum
\( J \) = Coupling constant  
Kg = Kilogram  
L = Liter  
\( \lambda_{\text{max}} \) = Wavelength at maximal absorption  
\( m \) = Multiplet (for NMR spectra)  
MeOH = Methanol  
mg = Milligram  
\( \mu \)g = Microgram  
min = Minute  
mL = Milliliter  
\( \mu \)L = Microliter  
\( \mu \)M = Micromolar  
mM = Millimolar  
MS = Mass spectrum  
MPLC = Medium Pressure Column Chromatography  
MW = Molecular weight  
m/z = Mass to charge ratio  
N/A = not available  
NI. = not indicated  
nm = Nanometer  
NMR = Nuclear Magnetic Resonances  
NO = Nitric oxide  
NOESY = Nuclear Overhauser Effect Spectroscopy  
\( \nu_{\text{max}} \) = Wave number at maximal absorption  
ppm = Part per million  
s = Singlet (for NMR spectra)  
Si-CC = Silica gel Column Chromatography  
t = Triplet (for NMR spectra)  
TLC = Thin Layer Chromatography  
UV-VIS = Ultraviolet and Visible spectrophotometry
CHAPTER I
INTRODUCTION

Inflammation is a general mechanism of body tissues for the prevention of invasion by infectious agents e.g., microbes into hosts. It is part of the biological response to damages from trauma or burns and involves antigen-antibody reactions and several inflammatory mediators such as nitric oxide, prostaglandins and other cytokines. Pain, swelling, warmth, redness and loss of function are significant symptoms of inflammation (Turner et al., 2014). Inflammation can be classified into 3 stages which are acute, subacute or chronic inflammation. Acute inflammation rapidly happens after infection. Blood vessels are dilated and capillary permeability is increased in order to facilitate the movement of plasma and white blood cells to the injured area. Subacute inflammation after happens after acute stage. Leukocyte and chemical mediators are released. These states can be activated by the temperature in the body (fever). The last stage of inflammation, called chronic inflammation, can occur several months after the initial injury. Fibrosis can be found after long-term injury. Chronic inflammation is the cause of various diseases including rheumatoid arthritis and osteoarthritis (Robert and Morrow, 2006). The World Health Organization (WHO) has pointed out that chronic inflammation can lead to a variety of diseases e.g. stroke, respiratory diseases, cardiovascular diseases and cancer (Tsai et al., 2019). Although Inflammation is an important process for the defense mechanism of the body, large scale inflammation or long-term inflammation may contribute to degeneration of the body. Therefore, in order to prevent inflammatory diseases, controlling of chemical mediators is necessary.

Nitric oxide is an important chemical mediator that plays a role in the inflammation process. It can be synthesized by neutrophils, monocytes and macrophages using the enzyme inducible nitric oxide synthase (iNOS) with oxygen and NADPH as co-factors and released into endothelial cells causing vasodilation. But large amount of nitric oxide can cause tissue to degenerate (Sharma et al., 2007). Inhibition of nitric oxide is a needful method to control inflammation (Vane and Botting, 1998). Anti-inflammatory drugs, either steroids or non-steroids (NSAIDS), have been used for
this purpose. However, long-term treatment can lead to various side effects (e.g., peptic ulcer, cataract and osteoporosis).

Many medicinal plants in traditional medicine have been used to treat inflammatory diseases, although there is little scientific evidence to support. Research study on the ability of extracts and chemical constituents of these plants to inhibit production of nitric oxide by macrophages is an alternative in the discovery and development of novel anti-inflammatory drugs for future use with minimal side effects and greater safety.

Family Capparaceae (or Capparidaceae) is a family of flowering plants consisting of 40-45 genera (700-900 species) distributed in tropical and subtropical regions. It is closely related to family Brassicaceae (Cruciferae) (Cronquist, 1981; Heywood, 1993; Mabberley, 1997). Several members of this plant family have been used as herbal drugs, food or cosmetics. Some species have displayed various biological activities such as anti-diabetic, antimicrobial, anticancer and anti-inflammatory (Bektas et al., 2012; Nabavi et al., 2016; Verma et al., 2013). *Capparis* is the largest genus in this family (consisting about 250 species). Major chemical constituents of plants in this genus are alkaloids, glucosinolates and isothiocynates. Some Capparis species are used in traditional medicine to cure inflammatory diseases e.g., rheumatism and cystitis). In Thailand, the roots of *Capparis microcantha* have been used as a component of Ya-Ha-Rak, a traditional Thai drug formula to treat fever symptoms (Palo et al., 2017). Extracts from several parts of *C. spinosa*, or caper bush, have been reported to possess several biological activities. Its fruit and leaf extracts showed antidiabetic activity in rats (Chen et al., 2017). The fruit extract also showed hypotensive effect, whereas both root and fruit extracts showed antimicrobial activity (Zhang and Ma, 2018). Extracts from a number of *Capparis* species including *C. spinosa* (Nabavi et al., 2016; Chen et al., 2017; Nabavi et al., 2016; Zhang and Ma, 2018), *C. ovata* (Bektas et al., 2012), *C. decidua* (Verma et al., 2013), *C. tomentosa* (Akoto et al., 2008) and *C. acutifolia* (Chen et al., 2017) were demonstrated to have anti-inflammatory activity.

*Maerua* is another genus of family Capparaceae. It consists of approximately 90 species (Chayamarit, 1991). Major chemical constituents of *Maerua* were reported
to be alkaloids, flavonoids, glucosinolates and isothiocyanates (Nobsathian et al., 2018). Members of this genus have been employed to treat several ailments such as fever, stomach ache, skin infection, diabetes, and urinary calculi. Biological activities of extracts from Maerua species have also been reported. Methanol extracts of *Maerua angolensis* and *M. pseudopetalosa* showed antimicrobial activity. Extracts from *M. crassifolia, M. angolensis* and *M. apetala* were reported as exhibiting anti-inflammatory activity (Lincy et al., 2014). Extracts from *M. siamensis* showed anti-inflammatory property by inhibition of albumin denaturation (Theanphong and Somwong, 2022).

Therefore, this study focused on *Capparis micracantha* and *Maerua siamensis*, two members of family Capparaceae used in traditional Thai medicine for treatment of diseases and symptoms associated with inflammation. Preliminary study of the extracts from the stems of *C. micracantha* and the roots *M. siamensis* showed their ability to inhibit nitric oxide production. Attempt to isolate their chemical constituents and evaluate their inhibitory activity on nitric oxide production might support the uses of these plants in traditional medicine and yield new anti-inflammatory drugs for the future, as well as providing chemotaxonomic information in the study of family Capparaceae.

The major objectives of this study were as follows.

1. To isolate and purify the chemical constituents from *Capparis micracantha* and *Maerua siamensis*.

2. To elucidate the chemical structures of the isolated compounds.

3. To examine inhibitory activity of these compounds on nitric oxide production in macrophages.
CHAPTER II
LITERATURE REVIEWS

2.1 Order Brassicales

Order Brassicales (or Capparales) is a group of dicotyledons in the APG III system, which consists of 17 families including Akaniaceae, Bataceae, Brassicaceae, Capparaceae, Caricaceae, Cleomaceae, Emblingiaceae, Gyrostemonaceae, Koeberliniaceae, Limnanthaceae, Moringaceae, Pentadiplandraceae, Resedaceae, Salvadoraceae, Setchellanthaceae, Tovariaceae and Tropaeolaceae (Hall et al., 2002). A characteristic of these families within the order is the production of indole glucosinolates, which can protect the plant itself from herbivore and parasitism and can sometimes be used as flavoring agent eliciting pungent taste (Simpson, 2019).

Within this order, family Brassicaceae is the largest with approximately 340 genera and 3,350 species that are economically important as food, such as many vegetables including broccoli, brussels sprouts, cauliflowers and cabbages, industrial crops, and ornamental plants. These families are distributed worldwide, but most of them are found in northern regions, around Mediterranean basin, south-western and central Asia. Although Capparaceae is related to the herbaceous Brassicaceae, some species can sometimes be big woody trees. These Capparaceae plants usually have elongate gynophore or androgynophore, many stamens and unilocular ovary in a parietal placentation (Al-Shehbaz et al., 2006; Bailey et al., 2006; Beilstein et al., 2008).

In the past, Cleomaceae used to be a genus (Cleome) within family Capparaceae. But in 2016, APG IV classification system has separated this genus into a new monophyletic family (Simpson, 2019). Cleome species have different characteristics from other plants in the family Capparaceae including being herbaceous, having dehiscent fruit with a replum and unilocular ovary. Recently, chemical constituents and DNA phylogeny of Brassicaceae and Capparaceae have been studied to provide their chemotaxonomic data.
2.2 Family Capparaceae

Capparaceae (or Capparidaceae) is a medium-size family in order Brassicales that is closely related to family Brassicaceae (Cruciferae). It consists of approximately 29 genera and 381 species. This family used to be divided into 2 subfamilies: Capparoideae and Cleomoideae. There are about 25 genera and 440 species within subfamily Capparoideae which are shrub or small tree bearing fleshy fruit (dehiscent or indehiscent). Subfamily Cleomoideae consisted of about 8 genera and 275 species which were herbaceous and had dehiscent fruits. Plants in this family are widely distributed in the tropical and subtropical regions (Kamel et al., 2009). In Thailand, three genera of this family have been recorded, including Capparis, Crateva and Maerua.

Plants in Capparaceae can be herb, shrub or, sometimes, woody tree. Their leaves are simple or palmately compound. The leaf arrangement is alternate or, rarely, opposite. The texture of leaves is glabrous or furnished with glands or glandular hairs. These leaves are either stipulate or exstipulate. The flower is solitary or in axillary or terminal inflorescence, of racemes or corymbbs. Flowers are bisexual or rarely unisexual, actinomorphic or zygomorphic. There are 4-8 sepals and 4-16 petals. The stamens are free, few to many. The ovary is superior, mostly borne on gynophore, or sessile. Ovules are few to many. Fruit is berry, drupe, nutlet or siliquiform. Seeds are often many (Kamel et al., 2009).

2.3 Genus Capparis

Capparis is a big genus in the family Capparaceae consisting of 250 species which are distributed worldwide in the tropical and subtropical regions. Their flowers have numerous stamens and a short or elongated androgynophore. Plants in this genus can strongly grow in adverse conditions (water stresses, photo-inhibition and high irradiance) (Cristina et al., 2006). Historically, several Capparis species have been used since ancient times as drug and food. For example, the roots of Capparis spinosa were consumed by Egyptians and Arabs to treat kidney disease, liver disease and stomach problem. The leaves of this plants were used to cure skin diseases and earache,
whereas the buds were used to treat disease of the spleen. Pickled flower buds, unripe fruits and shoots of some Capparis plants are usually stored in salt, vinegar and brine for used as appetizer (Tili et al., 2011). In Thailand, there are 17 species of the genus Capparis as listed below.

<table>
<thead>
<tr>
<th>No.</th>
<th>Name of species</th>
<th>Thai names</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Capparis acuminata Willd.</td>
<td>แมงซอ</td>
</tr>
<tr>
<td>2</td>
<td>C. acutifolia Sw. subsp. sabiaefolia Jacobs</td>
<td>ต้าผู้แม</td>
</tr>
<tr>
<td>3</td>
<td>C. diffusa Ridl.</td>
<td>หหมามเกี่ยวไก์</td>
</tr>
<tr>
<td>4</td>
<td>C. echinocarpa Pierre ex Gagnep.</td>
<td>เปียไก์</td>
</tr>
<tr>
<td>5</td>
<td>C. flavicans Kurz.</td>
<td>จับเลย</td>
</tr>
<tr>
<td>6</td>
<td>C. floribunda Wight.</td>
<td>เปลดเซาะ</td>
</tr>
<tr>
<td>7</td>
<td>C. glauca Wall.</td>
<td>หหมำทักลิ้น</td>
</tr>
<tr>
<td>8</td>
<td>C. grandis Linn. f.</td>
<td>ค่อนกล้อง</td>
</tr>
<tr>
<td>9</td>
<td>C. micracantha DC.</td>
<td>ดิ้งชี้</td>
</tr>
<tr>
<td>10</td>
<td>C. pranensis Jacobs.</td>
<td>เพ็ดดีดังงาม</td>
</tr>
<tr>
<td>11</td>
<td>C. pyrifolia Lamk.</td>
<td>หหมำห่อมกลิ้น</td>
</tr>
<tr>
<td>12</td>
<td>C. radula Gagnep.</td>
<td>หหมำด่า</td>
</tr>
<tr>
<td>13</td>
<td>C. sepiaria Linn.</td>
<td>หหมำว้าช่าง</td>
</tr>
<tr>
<td>14</td>
<td>C. siamensis Kurz.</td>
<td>ฟุ้งแกะ</td>
</tr>
<tr>
<td>15</td>
<td>C. tenera Dalz.</td>
<td>หหมำเล่ยแมว</td>
</tr>
<tr>
<td>16</td>
<td>C. thorelii Gagnep.</td>
<td>จับช่าง</td>
</tr>
<tr>
<td>17</td>
<td>C. zeylanica Linn.</td>
<td>สะแอะ</td>
</tr>
</tbody>
</table>

2.4 *Capparis micracantha* DC.

*Capparis micracantha* DC. is a shrub or small tree that can grows up to 2-6 meters. The plant can sometimes have spines on its stem, the length of spines is about 2-4 mm. The leaves are simple, 3-5 cm long and 9.4-24 cm wide. The shape of these leaves is ovate-lanceolate or oblong-lanceolate or oblong to elliptic. The leaves are glabrous and light green in color. Their petioles are 0.1-1 cm long. The flowers are
solitary, or in clusters of 1-7 flowers, with peduncles which are 1-2 cm in length. The shape of sepals is ovate to oblong, the petals are free, nearly boat-shaped or oblong to obovate, 3-7 mm wide and 10-22 long. The petals are white, with dark red, dark violet or brownish spots. The number of stamens is 20-35. The filaments are 18-30 mm long. The ovary is borne on a gynophore of 2-3.5 cm in length. Its fruit is simple, ovoid or rounded. The color of fruits is yellow, red, or black. (Chayamarit, 1991).

Figure 1. *Capparis micracantha* DC.
A) Flowers  B) Habit  C) Leaves
(Photos by L. Nonthalert.)
2.5 Genus *Maerua*

Genus *Maerua* comprises 90 species in family Capparaceae. Most of these plants are distributed in tropical Asia and Africa. There is only one *Maerua* species in Thailand (*M. siamensis*). Plants of this genus are either shrub or tree. Their leaves are compound, 3-folioate. The flowers are in clusters or sometimes solitary. The inflorescences are corymbose or racemose. The flower has 4 sepals separated at base but has no petal. The number of stamens is few to numerous (20-40). The gynophore is long, bearing an ovary with one locule and numerous ovules. The stigma is disc-shaped. The fruits are round and glabrous and the seeds are large, 1-3 in number (Chayamarit, 1991).

2.6 *Maerua siamensis* (Kurz) Pax

*Maerua siamensis* (Kurz) Pax, which is the only *Maerua* species found in Thailand, is a shrub or small tree, up to 5-10 meters in height. Its twig is glabrous, with palmately compound leaves of 3 leaflets (or, rarely, 4-5 leaflets). The leaf shape is ovate or oblong (1-3 cm wide and 5-7 cm long) The leaf base is obtuse or hastate, and the leaf apex is cuspidate. The flower has no petal but has 4 distinct greenish-white sepals. The flowers are in terminal or axillary inflorescence. Some flowers can singly bloom from leaf axil. Its roots have been used as herbal drug to treat cystitis, to relieve pain and inflammation (Chayamarit, 1991).
2.7 Chemical constituents of plants in the families Brassicaceae and Capparaceae and their biological activities

Many vegetable plants belong to family Brassicaceae. Examples are broccoli (*Brassica oleracea* var. *italica*), cabbage (*B. oleracea* var. *capitata*), cauliflower (*B. oleracea* var. *botrytis*), kohlrabi (*B. oleracea* var. *gongylodes*) and brussels sprouts (*B. oleracea* var. *gennifera*). Major constituents in this family are glucosinolates and their derivatives, which are beneficial to these plants by protecting them from pathogens and insects. During cutting, chopping or chewing the plant, plant enzymes (e.g., $\beta$-thioglucosidase or myrosinase) are released and can cause hydrolysis of the glucosinolates. The aglycones of these glucosinolates can be divided into three groups:
aliphatic, indole and aromatic (Mithen et al., 2010) (Figure 3). In addition to isothiocyanate, break-down products from glucosinolate can also be epithionitrile, thiocyanate, nitrile and oxazolidine-thione (Al-Gendy et al., 2010) (Figure 4).

Several indole phytoalexins in Brassicaceae displayed interesting biological activities (Vig et al., 2009). For example, brassinin (10) has been reported to be cytotoxic toward leukemic cancer cell line, to exhibit cancer chemopreventive activity (Mehta et al., 1995), to enhance apoptosis and inhibit the metastasis of human prostate cancer (PC-3) cells (Kim et al., 2015). 1-Methoxybrassinin (115), firstly isolated from Chinese cabbage, was reported to be anti-proliferative against human colon cancer (Caco-2) cells (Chripkova et al., 2014) and anticancer against T-Jurkat leukemic cells (Pilatova et al., 2005).

Tryptanthrin (143), isolated from the leaves of Strobilanthes cusia (family Acanthaceae), showed antiviral activity against human coronavirus NL63 (Tsai et al., 2020). This compound, which was also found in Isatis plants in Brassicaceae, showed anti-inflammatory activity through inhibition of cyclooxygenase-2 (Danz et al., 2001), and inhibitory effect on prostaglandin and leukotriene synthesis (Danz et al., 2002).

![Diagram of glucosinolate precursors](image)

**Figure 3.** Three types of glucosinolate based on their amino acid precursors (Mithen et al., 2010)
Many secondary metabolites isolated and identified from Brassicaceae plants are indole alkaloids, as shown in Table 1. For example, several plants in the genus *Isatis* have been used for treatment of influenza, common cold and infection in traditional Chinese medicine. Extracts from these plants were reported to demonstrate anti-inflammatory activity through inhibition of nitric oxide production (Yang et al., 2014), and antiviral effect against coxsackievirus B3 and influenza virus type A. Indole alkaloids from the roots of *Isatis indigodica* such as isatigotindolediosides C (73) and E (75) (Meng et al., 2017) showed antiviral activity against coxsackievirus B3, whereas (−)-R-2-(3-cyanomethyl-4-methoxy-1H-indol-7-yl)-2-(1H-indol3-yl) acetonitrile (19), (−)-R-2-(3-cyanomethyl-4-methoxy-1H-indol-7-yl)-2-(4-methoxy-1H-indol-3-yl) acetonitrile (20) and arvelexin (1) were active against influenza virus type A (Chen et al., 2012). Isatindigobisindolosides B (79), D (81) and F (83), isatibisindosulfonic acid B (67) and isatindosulfonic acid B (97) from the same plant displayed antiviral activity against both viruses (Meng et al., 2017).
Investigation of the roots of another *Isatis* species, *Isatis tinctoria*, revealed the presence of ten indole alkaloids, mostly belonging to the isatindigoside and isatisindigoticanine subtypes (Zhang et al., 2020). These alkaloids exhibited inhibitory effect on nitric oxide production (Zhang et al., 2019).

Families Capparaceae and Brassicaceae are closely associated according to their DNA phylogeny and chemical constituents. Many studies have reported that the major secondary metabolites in Capparaceae are flavonoids, phenolic acids, steroids, triterpenoids, alkaloids, fatty acids and glucosinolates. Break-down products of glucosinolates are similar to those found in Brassicaceae plants, especially indole alkaloids, for example, (+)-R-2-(4-hydroxy-2-oxo-2,3-dihydrobenzofuran-3-yl) acetonitrile (216) and (+)-S-2-(4-hydroxy-2-oxo-2,3-dihydrobenzofuran-3-yl) acetonitrile (217), isolated from *Capparis spinosa* (Capparaceae), are similar to indole-3-acetonitrile (54), 1-methoxy-indole-3-acetonitrile (Caulilexin C) (16) and indole-3-acetonitrile-6-O-β-D-glucopyranoside (56), found in Brassicaceae family.

The chemical constituents in genus *Capparis* possess various biological activities, for example, the triterpenoids simiarenol (210) and lupeol (207) and the plant sterol β-sitosterol (200), isolated from *C. dongvanensis*, showed α-glucosidase inhibitory activity (Khang et al., 2021). Ginkgetin (173), a biflavonoid found in *C. spinosa*, showed inhibitory effect on NF-κB activation (H.-F. Zhou et al., 2011). Several flavonoids and their glycosides from this genus exhibited antioxidant activity (Yahia et al., 2020). A triterpenoid, olean-12-en-3β,28-diol 3β-pentacosanoate (208), from *C. ovata* showed moderate anti-inflammatory activity (Gazioglu et al., 2020). Additionally, cappariloside A (152), firstly purified from *C. spinosa*, showed antiviral activity by inhibiting the replication of H1N1, H3N2, PIV3 and ADV viruses (Li et al., 2018).

The majority of chemical constituents reported from plants in the genus *Maerua* are phenolic compound, fatty acids, triterpenoids and steroids. A small number of alkaloids and flavonoids have also been found. Some of these compounds, e.g., capparilosides A (152) and B (153) from the leaves and twigs of *M. siamensis,*
exhibited larvicidal activity against the larvae of *Aedes aegypti* mosquito (Nobsathian et al., 2018).

The distribution of chemical constituents in the family Brassicaceae and genera *Capparis* and *Maerua* of family Capparaceae is presented in Tables 1, 2 and 3.

**Table 1. Distribution of Indole alkaloids family Brassicaceae**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Source</th>
<th>Plant part</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arvelexin (1)</td>
<td><em>Isatis indigotica</em></td>
<td>Roots</td>
<td>Yang et al. (2014)</td>
</tr>
<tr>
<td></td>
<td><em>Thlaspi arvense</em></td>
<td>Leaves</td>
<td>Pedras et al. (2003)</td>
</tr>
<tr>
<td>Benzocamalexin (2)</td>
<td><em>Thellungiella halophila</em></td>
<td>Aerial parts</td>
<td>Pedras et al. (2009)</td>
</tr>
<tr>
<td>Biswasalexin A1 (3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biswasalexin A2 (4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brassicanal B (6)</td>
<td><em>B. campestris</em></td>
<td>Ni.</td>
<td>(Monde et al., 1991a)</td>
</tr>
<tr>
<td>Brassicanal C (7)</td>
<td><em>B. oloracea</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>I. tinctoria</em></td>
<td>Roots</td>
<td>Zhang et al. (2022)</td>
</tr>
<tr>
<td>Brassilexin (9)</td>
<td><em>B. juncea</em></td>
<td>Leaves</td>
<td>Devys et al., 1998</td>
</tr>
<tr>
<td>Brassinin (10)</td>
<td><em>B. campestris</em></td>
<td>Ni.</td>
<td>Takasugi et al. (1987)</td>
</tr>
<tr>
<td>Camalexin (13)</td>
<td><em>Camelina sativa</em></td>
<td>Leaves</td>
<td>Browne et al. (1991)</td>
</tr>
<tr>
<td>Compound</td>
<td>Source</td>
<td>Plant part</td>
<td>Reference</td>
</tr>
<tr>
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</tr>
<tr>
<td>Caulilexin A (14)</td>
<td><em>B. oleracea</em></td>
<td>NI.</td>
<td><em>Pedras et al.</em> (2006)</td>
</tr>
<tr>
<td>Caulilexin B (15)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-[Cyano(3-indolyl)methylene]-3-indolone (18)</td>
<td><em>I. tinctoria</em></td>
<td>Whole plants</td>
<td><em>Ahmad et al.</em> (2008)</td>
</tr>
<tr>
<td>(−)-R-2-(3-Cyanomethyl-4-methoxy-1H-indol-7-yl)-2-(1H-indol3-yl) acetonitrile (19)</td>
<td><em>I. indigotica</em></td>
<td>Roots</td>
<td><em>Chen et al.</em> (2012)</td>
</tr>
<tr>
<td>(−)-R-2-(3-Cyanomethyl-4-methoxy-1H-indol-7-yl)-2-(4-methoxy-1H-indol-3-yl) acetonitrile (20)</td>
<td><em>I. indigotica</em></td>
<td>Roots</td>
<td><em>Chen et al.</em> (2012)</td>
</tr>
<tr>
<td>Cyclobassinin (21)</td>
<td><em>B. campestris</em></td>
<td>NI.</td>
<td><em>Takasugi et al.</em> (1987)</td>
</tr>
<tr>
<td>Cyclobassinin sulfoxide (22)</td>
<td><em>B. juncea</em></td>
<td>NI.</td>
<td><em>Devys et al.</em> (1990)</td>
</tr>
<tr>
<td>Cyclobassinone (23)</td>
<td><em>B. oleracea</em> var <em>gongylodes</em></td>
<td>Stems</td>
<td><em>Gross et al.</em> (1994)</td>
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<tr>
<td>Dehydrocyclobassinin (24)</td>
<td><em>B. napus</em></td>
<td>Roots</td>
<td><em>Pedras et al.</em> (2008a)</td>
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<td>9α,13α-Dihydroxylisopropylidenyl-isatisine A (25)</td>
<td><em>I. tinctoria</em></td>
<td>Roots</td>
<td><em>Hong et al.</em> (2019)</td>
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<tr>
<td>(+)-(S)-2-(3,4-Dihydroxy-2-oxoindolin-3-yl) acetonitrile (26)</td>
<td><em>I. indigotica</em></td>
<td>Roots</td>
<td><em>Chen et al.</em> (2012)</td>
</tr>
<tr>
<td>Dioxibrassinin (27)</td>
<td><em>B. oleracea</em></td>
<td>NI.</td>
<td><em>Monde et al.</em> (1991a)</td>
</tr>
<tr>
<td>Compound</td>
<td>Source</td>
<td>Plant part</td>
<td>Reference</td>
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<td>------------------------------------------------------------------------</td>
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<tr>
<td>2,2-Di-(3-indolyl)-3-indolone (28)</td>
<td><em>I. indigotica</em></td>
<td>Leaves</td>
<td>Yang and Bao (2020)</td>
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<td></td>
<td><em>I. tinctoria</em></td>
<td>Roots</td>
<td>Zhang et al. (2022)</td>
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<td>Epi-glucoisatisin (29)</td>
<td><em>I. tinctoria</em></td>
<td>Roots</td>
<td>Frechard et al. (2001)</td>
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<tr>
<td></td>
<td><em>I. tinctoria</em></td>
<td>Whole plants</td>
<td>Ahmad et al. (2008)</td>
</tr>
<tr>
<td>Epi-isatidifolioside A (30)</td>
<td><em>I. indigotica</em></td>
<td>Leaves</td>
<td>Guo et al. (2020)</td>
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<tr>
<td>Epi-isatidifolioside B (31)</td>
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<tr>
<td>Epiphatanthrin A (32)</td>
<td><em>I. indigotica</em></td>
<td>Roots</td>
<td>Liu et al. (2016)</td>
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<tr>
<td>Erucalexin (33)</td>
<td><em>Erucastrum gallicum</em></td>
<td>Leaves</td>
<td>(Pedras et al., 2006)</td>
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<td>Glucobrassicin (34)</td>
<td><em>B. oleracea</em></td>
<td>Ni.</td>
<td>Gmelin et al. (1960)</td>
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<td>Glucoisatisin (35)</td>
<td><em>I. tinctoria</em></td>
<td>Seeds</td>
<td>Antoine et al. (2001)</td>
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<tr>
<td>2-[(4-β-D-Glucopyranosyloxy)-1H-indol-3-yl] acetonitrile (36)</td>
<td><em>I. tinctoria</em></td>
<td>Roots</td>
<td>Zhang et al. (2022)</td>
</tr>
<tr>
<td>β-D-Glucopyranosyl indole-3-carboxylic acid (37)</td>
<td><em>I. tinctoria</em></td>
<td>Roots</td>
<td>Zhang et al. (2022)</td>
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<td>Homobrassinin (38)</td>
<td><em>B. oleracea</em></td>
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<td>(Mehta et al., 1995)</td>
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<td>3'-Hydroxyepiglucoisatisin (39)</td>
<td><em>I. tinctoria</em></td>
<td>Whole plants</td>
<td>Ahmad et al. (2008)</td>
</tr>
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<td></td>
<td><em>I. tinctoria</em></td>
<td>Ni.</td>
<td>Antoine et al. (2001)</td>
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<tr>
<td>4-Hydroxyglucobrassicin (40)</td>
<td><em>B. oleracea</em></td>
<td>Ni.</td>
<td>Truscott et al. (1983)</td>
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<tr>
<td>3'-Hydroxyglucoisatisin (41)</td>
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<td>Seeds</td>
<td>Antoine et al. (2001)</td>
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<tr>
<td>Compound</td>
<td>Source</td>
<td>Plant part</td>
<td>Reference</td>
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<tr>
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<td>(E)-2-(4-Hydroxy-2-oxoindol-3-ylidene) acetonitrile (42)</td>
<td><em>I. indigotica</em></td>
<td>Roots</td>
<td>Chen et al. (2012)</td>
</tr>
<tr>
<td>(−)-(R)-2-(4-Hydroxy-2-oxoindol-3-yl) acetamide (43)</td>
<td><em>I. indigotica</em></td>
<td>Roots</td>
<td>Chen et al. (2012)</td>
</tr>
<tr>
<td>(−)-(R)-2-(4-Hydroxy-2-oxoindol-3-yl) acetonitrile (44)</td>
<td></td>
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<td>(+)-(S)-2-(3-Hydroxy-4-methoxy-2-oxoindol-3-yl) acetamide (45)</td>
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<td>(−)-(S)-2-(3-Hydroxy-4-methoxy-2-oxoindol-3-yl) acetonitrile (46)</td>
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<tr>
<td>(+)-(S)-2-[7-[1-(4-Hydroxyphenyl)-ethyl]-4-methoxy-1H-indol-3-yl]acetonitrile (47)</td>
<td><em>I. indigotica</em></td>
<td>Roots</td>
<td>Chen et al. (2012)</td>
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<tr>
<td>(−)-(2R,3R)-3-Hydroxy-2H-pyrrolo[2,3-b]indolo[5,5a,6-b,a]quinazoline-9(8H),7'-dione (49)</td>
<td><em>I. indigotica</em></td>
<td>Roots</td>
<td>Chen et al. Chen et al. (2012)</td>
</tr>
<tr>
<td>(+)-(2S,3S)-3-Hydroxy-2H-pyrrolo[2,3-b]indolo[5,5a,6-b,a]quinazoline-9(8H),7'-dione (50)</td>
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<td>Indigotin (51)</td>
<td><em>I. indigotica</em></td>
<td>Leaves, Roots</td>
<td>Zou (2007)</td>
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<tr>
<td>Indirubin (52)</td>
<td><em>Isatidis folium</em></td>
<td>Leaves</td>
<td>Lu et al. (2012)</td>
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<td>3-Indoleacetic acid (53)</td>
<td><em>B. oleracea var. capitata</em></td>
<td>Heads</td>
<td>Weller et al. (1953)</td>
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<tr>
<td>Compound</td>
<td>Source</td>
<td>Plant part</td>
<td>Reference</td>
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<td>Indole-3-acetonitrile-2-S-β-D-glucopyranoside (55)</td>
<td>I. indigotica</td>
<td>Roots</td>
<td>Yang et al. (2014)</td>
</tr>
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<td>Indole-3-acetonitrile-6-O-β-D-glucopyranoside (56)</td>
<td>I. indigotica</td>
<td>Roots</td>
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<tr>
<td>Indole-3-acetonitrile-4-methoxy-2-S-β-D-glucopyranoside (57)</td>
<td>I. indigotica</td>
<td>Roots</td>
<td>Yang et al. (2014)</td>
</tr>
<tr>
<td>1H-Indole-3-carboxylic acid (58)</td>
<td>I. tinctoria</td>
<td>Roots</td>
<td>Zhang et al. (2022)</td>
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<td>3-Indoleformic acid (59)</td>
<td>I. indigotica</td>
<td>Roots</td>
<td>Yang et al. (2014)</td>
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<td></td>
<td>I. tinctoria</td>
<td>Roots</td>
<td>Zhang et al. (2022)</td>
</tr>
<tr>
<td>2-(1H-Indol-2-yl)-6-methoxy-4(3H)quinazolinone (60)</td>
<td>I. tinctoria</td>
<td>Roots</td>
<td>Zhang et al. (2019)</td>
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<td>(2’)-2-(1H-Indol-3-ylmethylidene)-1,2-dihydro-3H-indol-3-one (61)</td>
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<td>Roots</td>
<td>Chen et al. (2012)</td>
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<tr>
<td>2-(1H-Indol-2-yl)-4(3H)-quinolinone (62)</td>
<td>I. tinctoria</td>
<td>Roots</td>
<td>Zhang et al. (2019)</td>
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<td>Isalexin (63)</td>
<td>B. napus var. rapifera</td>
<td>Tubers</td>
<td>Pedras et al. (2004)</td>
</tr>
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<td>Isatan A (64)</td>
<td>I. indigotica</td>
<td>Roots</td>
<td>Oberthur et al. (2004)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Whole plants</td>
<td>Oberthur et al. (2004)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ni.</td>
<td>Ahmad et al. (2008)</td>
</tr>
<tr>
<td>Isatan B (65)</td>
<td>I. tinctoria</td>
<td>Ni.</td>
<td>Oberthur et al. (2004)</td>
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<td>Isatibisindosulfonic acid A 3-O-β-D-glucopyranoside (66)</td>
<td>I. indigotica</td>
<td>Roots</td>
<td>Meng et al. (2017)</td>
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<tr>
<td>Isatibisindosulfonic acid B (67)</td>
<td>I. indigotica</td>
<td>Ni.</td>
<td>Oberthur et al. (2004)</td>
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<tr>
<td>Compound</td>
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<td>Plant part</td>
<td>Reference</td>
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<tr>
<td>Isatidifoliumoside A (68)</td>
<td><em>I. indigotica</em></td>
<td>Leaves</td>
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<td>Isatidifoliumoside B (69)</td>
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<td>Isatidifoliumoside D (70)</td>
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<td>Isatigotindoleidoside A (71)</td>
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<td>Isatigotindoleidoside C (73)</td>
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<td>Isatigotindoleidoside D (74)</td>
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<td>Isatigotindoleidoside E (75)</td>
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<td>Isatigotindoleidoside F (76)</td>
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<td>Isatin (77)</td>
<td><em>I. indigotica</em></td>
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<td>Isatindigobisindoloside A (78)</td>
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<td>Roots</td>
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<td>Isatindigobisindoloside D (81)</td>
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<td>Isatindigobisindoloside E (82)</td>
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<td>Isatindigobisindoloside F (83)</td>
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<td>Isatindigobisindoloside G (84)</td>
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<td>Isatindigodiphindoside (85)</td>
<td><em>I. indigotica</em></td>
<td>Roots</td>
<td>Meng et al. (2018)</td>
</tr>
<tr>
<td>Isatindigoside D (86)</td>
<td><em>I. tinctoria</em></td>
<td>Roots</td>
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<td>Isatindigoside F (87)</td>
<td><em>I. tinctoria</em></td>
<td>Roots</td>
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<td>Isatindigoside G (88)</td>
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<td>Isatindigoside H (89)</td>
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<td>Roots</td>
<td>Zhang et al. (2020)</td>
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<td>Isatindigoside I (90)</td>
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<td>Isatindigoside J (91)</td>
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<td>Isatindigoside K (92)</td>
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<td>Isatindigoside L (93)</td>
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<td>Plant part</td>
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<td>Isatindigoside M (94)</td>
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<td>Roots</td>
<td>Zhang et al. (2022)</td>
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<td>Isatindigotindoloside B (95)</td>
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<td>Isatindosulfonic acid B (97)</td>
<td><em>I. indigotica</em></td>
<td>Roots</td>
<td>Meng et al. (2017)</td>
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<td>Isatindosulfonic acid C (98)</td>
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<td>Isatindosulfonic acid D (99)</td>
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<td>Isatindosulfonic acid E (100)</td>
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<td>Isatindosulfonic acid F (101)</td>
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<td>Isatisindigoticanine A (102)</td>
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<td>Roots</td>
<td>Zhang et al. (2019)</td>
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<td>Isatisindigoticanine F (103)</td>
<td><em>I. tinctoria</em></td>
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<td>Isatisindigoticanine H (105)</td>
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<td>Isatisindigoticanine J (107)</td>
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<td>Isatisindigoticanine K (108)</td>
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<td>Isatisindigoticanine L (109)</td>
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<td>Isatisindigoticanine M (110)</td>
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<td>Roots</td>
<td>Zhang et al. (2022)</td>
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<td>Isatisindigoticanine N (111)</td>
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<td>Isatisine A (112)</td>
<td><em>I. indigotica</em></td>
<td>Leaves</td>
<td>Liu et al. (2007)</td>
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<td>1-Methoxybrassinin A (113)</td>
<td><em>B. oleracea</em> var. capitata</td>
<td>Nl.</td>
<td>Monde et al. (1991b)</td>
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<tr>
<td>1-Methoxybrassinin B (114)</td>
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<td>1-Methoxybrassinin (115)</td>
<td><em>B. campestris</em> var. pekinensis</td>
<td>Nl.</td>
<td>Takasugi et al. (1987)</td>
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<td>4-Methoxybrassinin (116)</td>
<td><em>B. oleracea</em></td>
<td>Nl.</td>
<td>Monde et al. (1990)</td>
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<td>Plant part</td>
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<tr>
<td>1-Methoxybrassinin (117)</td>
<td><em>B. oleracea</em> var. botrytis</td>
<td>Ni.</td>
<td>Pedras et al. (2006)</td>
</tr>
<tr>
<td>6-Methoxycamalexin (118)</td>
<td><em>Capsella bursapastoris</em></td>
<td>Leaves</td>
<td>Jimenez et al. (1997)</td>
</tr>
<tr>
<td>4-Methoxycyclobrassinon (119)</td>
<td><em>B. napus</em></td>
<td>Roots</td>
<td>Pedras (2008b)</td>
</tr>
<tr>
<td>4-Methoxycyclobrassinin (120)</td>
<td><em>B. oleracea</em></td>
<td>Ni.</td>
<td>Truscott et al. (1983)</td>
</tr>
<tr>
<td>4-Methoxyglucobrassicin (121)</td>
<td><em>B. oleracea</em></td>
<td>Ni.</td>
<td>Yang et al. (2014)</td>
</tr>
<tr>
<td>4-Methoxy-3-indoleacetic acid (122)</td>
<td><em>I. indigotica</em></td>
<td>Roots</td>
<td>Yang et al. (2014)</td>
</tr>
<tr>
<td>N-Methoxy-indole-3-acetonitrile-2-S-β-D-glucopyranoside (123)</td>
<td><em>I. indigotica</em></td>
<td>Roots</td>
<td>Yang et al. (2014)</td>
</tr>
<tr>
<td>1-Methoxy-3-indoleformic acid (124)</td>
<td><em>I. tinctoria</em></td>
<td>Roots</td>
<td>Zhang et al. (2022)</td>
</tr>
<tr>
<td>1-Methoxy-3-indoleformic acid (125)</td>
<td><em>I. indigotica</em></td>
<td>Roots</td>
<td>Yang et al. (2014)</td>
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<tr>
<td>1-Methoxyspirobrassinin (126)</td>
<td><em>B. oleracea</em></td>
<td>Stems</td>
<td>Gross et al. (1994)</td>
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<tr>
<td>1-Methoxyspirobrassinol (127)</td>
<td><em>Raphanus sativus</em> var. hortensis</td>
<td>Roots</td>
<td>Monde et al. (1995)</td>
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<tr>
<td>(2R,3R)-1-Methoxyspirobrassinol methyl ether (128)</td>
<td><em>Wasabia japonica</em></td>
<td>Ni.</td>
<td>Somei et al. (2001)</td>
</tr>
<tr>
<td>1-Methylbenzocamalexin (129)</td>
<td><em>B. oleracea</em></td>
<td>Ni.</td>
<td>Pedras et al. (2010)</td>
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<tr>
<td>1-Methyl-6′-cyanobenzocamalexin (130)</td>
<td><em>B. oleracea</em></td>
<td>Ni.</td>
<td>Pedras et al. (2010)</td>
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<tr>
<td>Methyl-1H-methoxyindole-3-carboxylate (131)</td>
<td><em>W. japonica</em></td>
<td>Ni.</td>
<td>Pedras et al. (1998)</td>
</tr>
<tr>
<td>Methyl quindoline-11-carboxylate (132)</td>
<td><em>I. indigotica</em></td>
<td>Leaves</td>
<td>Yang and Bao (2020)</td>
</tr>
<tr>
<td>Phaitanthrin A (133)</td>
<td><em>I. indigotica</em></td>
<td>Roots</td>
<td>Liu et al. (2016)</td>
</tr>
<tr>
<td>Phaitanthrin D (134)</td>
<td><em>I. indigotica</em></td>
<td>Leaves</td>
<td>Yang and Bao (2020)</td>
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<tr>
<td>Compound</td>
<td>Source</td>
<td>Plant part</td>
<td>Reference</td>
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<td>Phaius mishensis</td>
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<td>Jao et al. (2008)</td>
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<td>Rapalexin A (135)</td>
<td>B. napus</td>
<td>Leaves</td>
<td>Pedras et al. (2007)</td>
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<tr>
<td>Rutalexin (137)</td>
<td>B. napus var. rapifera</td>
<td>Tubers</td>
<td>Pedras et al. (2004)</td>
</tr>
<tr>
<td>Sinalbin A (138)</td>
<td>Sinapis alba</td>
<td>Leaves</td>
<td>Pedras et al. (2000)</td>
</tr>
<tr>
<td>Sinalbin B (149)</td>
<td>S. alba</td>
<td>NL.</td>
<td>Soledade et al. (1997)</td>
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<tr>
<td>(S)-Spirobrassinin (141)</td>
<td>Rhaphanus sativus var. hortensis</td>
<td>NL.</td>
<td>Takasugi et al. (1987)</td>
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<tr>
<td>Sulfogluco brassicin (142)</td>
<td>L. tinctoria</td>
<td>Whole plants</td>
<td>Ahmad et al. (2008)</td>
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<tr>
<td>Tryptanthrin (143)</td>
<td>L. indigotica</td>
<td>Leaves</td>
<td>Wei et al. (2019)</td>
</tr>
<tr>
<td></td>
<td>L. tinctoria</td>
<td>Roots</td>
<td>Speranza et al. (2020)</td>
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<tr>
<td>Wasalexin A (144)</td>
<td>W. japonica</td>
<td>NL.</td>
<td>Pedras et al. (1999)</td>
</tr>
<tr>
<td>Wasalexin B (146)</td>
<td></td>
<td></td>
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</tbody>
</table>

NI. refers to “not indicated”.

Arvelexin (1); R$_1$ = H, R$_2$ = OCH$_3$, R$_3$ = H
1-Methoxy-indole-3-acetonitrile (Caulilexin C) (16); R$_1$ = OCH$_3$, R$_2$ = H, R$_3$ = H
2-[(4-β-D-Glucopyranosyloxy)-1-indol-3-yl] acetonitrile (36); R$_1$ = H, R$_2$ = O-Glu, R$_3$ = H
Indole-3-acetonitrile (54); R$_1$ = H, R$_2$ = H, R$_3$ = H
Indole-3-acetonitrile-6-O-β-D-glucopyranoside (56); R$_1$ = H, R$_2$ = H, R$_3$ = O-Glu
Isatindigotindoloside B (97); R$_1$ = OCH$_3$, R$_2$ = H, R$_3$ = O-Glu
Benzocamalexin (2); \( R_1 = R_2 = H \)
1-Methylbenzocamalexin (128); \( R_1 = \text{CH}_3, R_2 = H \)
1-Methyl-6'-cyanobenzocamalexin (129); \( R_1 = \text{CH}_3, R_2 = \text{CN} \)
1-Methyl-6'-methoxybenzocamalexin (130); \( R_1 = \text{CH}_3, R_2 = \text{OCH}_3 \)
Brassicanal A (5)

Brassicanal B (6)

Brassicanal C (7)

Brassicanate A (8); R₁ = H, R₂ = SCH₃, R₃ = OCH₃

β-D-Glucopyranosyl indole-3-carboxylic acid (37); R₁ = R₂ = H, R₃ = O-Glu

1H-Indole-3-carboxylic acid (58); R₁ = R₂ = H, R₃ = OH

3-Indoleformic acid (59); R₁ = H, R₂ = SCH₃, R₃ = OH

Isatindigoside M (97); R₁ = OCH₃, R₂ = H, R₃ = O-Glu

Methyl-1H-methoxyindole-3-carboxylate (131); R₁ = OCH₃, R₂ = H, R₃ = OCH₃

1-Methoxy-3-indoleformic acid (124); R₁ = OCH₃, R₂ = H, R₃ = OH

Brassilexin (9) R = H

Sinalexin (140) R = OCH₃

Brassinin (10); R₁ = S, R₂ = SCH₃

Brassitin (11); R₁ = O, R₂ = SCH₃

Caulilexin B (15); R₁ = O, R₂ = H
(−)-R·2·[Cyano(3-indolyl)methylene]-3-indolone (18)

(−)-R·2·(3-Cyanomethyl-4-methoxy-1H-indol-7-yl)-2·(1H-indol3-yl) acetonitrile (19); R= H

(−)-R·2·(3-Cyanomethyl-4-methoxy-1H-indol-7-yl)-2·(4-methoxy-1H-indol3-yl) acetonitrile (20); R= OCH₃
Cyclobrassinin (21); R₁ = H, R₂ = SCH₃
Cyclobrassinin sulfoxide (22); R₁ = H, R₂ = S(O)CH₃
Sinalbin A (138); R₁ = OCH₃, R₂ = S(O)CH₃
Sinalbin B (139); R₁ = OCH₃, R₂ = SCH₃

Dehydrocyclobrassinin (24); R₁ = H
4-Methoxydehydrocyclobrassinin (121); R₁ = OCH₃

Cyclobrassincne (23); R = H
4-Methoxycyclobrassinone (119); R = OCH₃

9α,13α-Dihydroxylisopropylidenyl-isatisine A (25)

(+)-(S)-2-(3,4-Dihydroxy-2-oxoindolin-3-yl) acetonitrile (26); R₁ = OH, R₂ = CN
(−)-(R)-2-(4-Hydroxy-2-oxoindolin-3-yl) acetamide (43); R₁ = H, R₂ = CONH₂
(−)-(R)-2-(4-Hydroxy-2-oxoindolin-3-yl) acetonitrile (45); R₁ = H, R₂ = CN
Epiglucoisatisin (29); R= H, S-configuration
Glucoisatisin (35); R= H, R-configuration
3'-Hydroxyepiglucoisatisin (39); R= OH

Dioxibrassinin (27)

2,2-Di-(3-indolyl)-3-indolone (28)

Epiisatidifoliumoside A (30)

Epiisatidifoliumoside B (31)

Epiphaitanthrin A (32)
Erucalexin (33)

Glucobrassicin (34); \( R_1 = R_2 = H \)
4-Hydroxyglucobrassicin (40); \( R_1 = H, R_2 = OH \)
4-Methoxyglucobrassicin (122); \( R_1 = H, R_2 = OCH_3 \)

Homobrassinin (38)

(\( E \))-2-(4-Hydroxy-2-oxoindolin-3-ylidene) acetonitrile (42)

(+)-(S)-2-(3-Hydroxy-4-methoxy-2-oxoindolin-3-yl) acetamide (45); \( R = CONH_2 \)
(+)-(S)-2-(3-Hydroxy-4-methoxy-2-oxoindolin-3-yl) acetonitrile (46); \( R = CN \)

(+)-(S)-2-[7-[1-(4-Hydroxyphenyl)-ethyl]-4-methoxy-1H-indol-3-yl] acetonitrile (47)
(2E)-N-(2-Hydroxyphenyl)-2-(1-hydroxy-3-oxoindolin-2-ylidene) acetamide (48)

(-)-(2R,3R)-3-Hydroxy-2H-pyrrolo[2,3-b]indolo[5,5a,6-b,a]quinazoline-9(8H),7'-dione (49)

(+)-(2S,3S)-3-Hydroxy-2H-pyrrolo[2,3-b]indolo[5,5a,6-b,a]quinazoline-9(8H),7'-dione (50)

3-Indoleacetic acid (53); R= H
4-Methoxy-3-indoleacetic acid (122); R= OCH₃

Indigotin (51)
Indirubin (52)
Indole-3-acetonitrile (54)
Indole-3-acetonitrile-2-S-β-D-glucopyranoside (55); \( R_1 = R_2 = H \)

Indole-3-acetonitrile-4-methoxy-2-S-β-D-glucopyranoside (57); \( R_1 = H, R_2 = \text{OCH}_3 \)

N-Methoxy-indole-3-acetonitrile-2-S-β-D-glucopyranoside (123); \( R_1 = \text{OCH}_3, R_2 = H \)

2-\{1H-Indol-2-yl\}-6-methoxy-4(3H)-quinazolinone (60)

2-\{1H-Indol-2-yl\}-6-methoxy-4(3H)-quinazolinone (60)

(Z)-2-\{1H-Indol-3-yl-methylidene\}-1,2-dihydro-3H-indol-3-one (61) 2-\{1H-Indol-2-yl\}-4(3H)-quinolinone (62)
Isalexin (63)

Isatan A (64)

Isatan B (65)

Isatibisindosulfonic acid A 3-O-β-D-glucopyranoside (66)

Isatibisindosulfonic acid B (67)

Isatidifoliumoside A (68)

Isatidifoliumoside B (69)

Isatidifoliumoside D (70)
Isatigotindoleidoside A (71); R= OCH$_3$
Isatigotindoleidoside B (72); R= H

Isatigotindoleidoside C (73)

Isatigotindoleidoside D (74)

Isatigotindoleidoside E (75)

Isatigotindoleidoside F (76)

Isatin (77)

Isatindigobisindoloside A (78)

Isatindigobisindoloside B (79)
Isatindigobisindoloside C (80)
Isatindigoside J (91)

Isatindigobisindoloside D (81)
Isatindigoside H (93)

Isatindigobisindoloside E (82)
Isatindigobisindoloside F (83)

Isatindigobisindoloside G (84)
Isatindigodiphindoside (85)
Isatindigoside D (86)

Isatindigoside F (87)
Isatindigoside G (88)

Isatindigoside I (90)
Isatindigoside K (92)

Isatindigoside L (93)
Isatindolienanoside A (96)
Isatin dosulfonic acid B (97)

Isatin dosulfonic acid C (98)

Isatin dosulfonic acid D (99); $R_1 = \text{OCH}_3$, $R_2 = \text{CN}$

Isatin dosulfonic acid D (101); $R_1 = \text{H}$, $R_2 = \text{COOH}$

Isatinindigoticanine A (102)

Isatinindigoticanine F (103)

Isatinindigoticanine G (104)
Isatisindigoticanine H (105)

Isatisindigoticanine I (106)

Isatisindigoticanine J (107)

Isatisindigoticanine K (108)

Isatisindigoticanine M (110)

Isatisindigoticanine N (111)
1-Methoxybrassinin A (113)

1-Methoxybrassinin B (114)

Isatisine A (112)

1-Methoxybrassinin (115); R₁ = OCH₃, R₂ = H, R₃ = S
4-Methoxybrassinin (116); R₁ = H, R₂ = OCH₃, R₃ = S
1-Methoxybrassitin (117); R₁ = OCH₃, R₂ = H, R₃ = O

6-Methoxycamalexin (118)

1-Methoxyspirobrassinin (125)

1-Methoxyspirobrassinol (126)
(2R,3R)-1-Methoxyspirobrassinol methyl ether (127)

Methyl quindoline-11-carboxylate (132)

Phaitanthrin A (133)

Phaitanthrin D (134)

Rapalexin A; R= H (135)

Rapalexin B; R= OH (136)

Rutalexin (137)
Table 2. Chemical constituents of plants in the genus *Capparis*

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Source</th>
<th>Plant part</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alkaloids</strong></td>
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<tr>
<td>14-N-Acetylisocodonocarpine (146)</td>
<td><em>Capparis decidua</em></td>
<td>Root barks</td>
<td>Forster <em>et al.</em> (2016)</td>
</tr>
<tr>
<td>15-N-Acetylccapparisine (147)</td>
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<tr>
<td>Berberine (148)</td>
<td><em>C. dongvanensis</em></td>
<td>Leaves</td>
<td>Khang <em>et al.</em> (2021)</td>
</tr>
<tr>
<td>Cadabicine (149)</td>
<td><em>C. decidua</em></td>
<td>Nl.</td>
<td>Ahmad <em>et al.</em> (1986)</td>
</tr>
<tr>
<td>Compounds</td>
<td>Source</td>
<td>Plant part</td>
<td>Reference</td>
</tr>
<tr>
<td>---------------------------------</td>
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<td>-------------------------</td>
</tr>
<tr>
<td>Capparidisine (150)</td>
<td><em>C. decidua</em></td>
<td>Nl.</td>
<td>Ahmad et al. (1986)</td>
</tr>
<tr>
<td>Capparidisinine (151)</td>
<td><em>C. decidua</em></td>
<td>Root barks</td>
<td>Forster et al. (2016)</td>
</tr>
<tr>
<td>Cappariloside A (152)</td>
<td><em>C. spinosa</em></td>
<td>Fruits</td>
<td>Calis et al. (1999)</td>
</tr>
<tr>
<td>Cappariloside B (153)</td>
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<tr>
<td>Capparin A (154)</td>
<td><em>C. sinaica</em></td>
<td>Fruits</td>
<td>Zhou et al. (2010)</td>
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<td>Capparin B (155)</td>
<td><em>C. himalayensis</em></td>
<td>Nl.</td>
<td>Li et al. (2008)</td>
</tr>
<tr>
<td>Capparisine (156)</td>
<td><em>C. decidua</em></td>
<td>Root barks</td>
<td>Ahmad et al. (1986)</td>
</tr>
<tr>
<td>Capparisine A (157)</td>
<td><em>C. spinosa</em></td>
<td>Fruits</td>
<td>Yang et al. (2010)</td>
</tr>
<tr>
<td>Capparisine B (158)</td>
<td></td>
<td></td>
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<tr>
<td>Capparisine C (159)</td>
<td></td>
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<tr>
<td>Capparispine (160)</td>
<td><em>C. spinosa</em></td>
<td>Roots</td>
<td>Fu et al. (2008)</td>
</tr>
<tr>
<td>Codonocarpine (161)</td>
<td><em>C. decidua</em></td>
<td>Root barks</td>
<td>Forster et al. (2016)</td>
</tr>
<tr>
<td>Flazin (162)</td>
<td><em>C. spinosa</em></td>
<td>Fruits</td>
<td>Zhou et al. (2010)</td>
</tr>
<tr>
<td>4-Hydroxy-1H-indole-3-carboxaldehyde (163)</td>
<td><em>C. spinosa</em></td>
<td>Aerial parts</td>
<td>Zhou et al. (2010)</td>
</tr>
<tr>
<td>2-(5-Hydroxymethyl-2-formylpyrrol-1-yl) propionic acid lactone (164)</td>
<td><em>C. spinosa</em></td>
<td>Fruits</td>
<td>(Yang et al., 2010)</td>
</tr>
<tr>
<td>3-Hydroxy-3-methyl-4-methoxyoxindole (165)</td>
<td><em>C. tomentosa</em></td>
<td>Roots</td>
<td>Akoto et al. (2008)</td>
</tr>
<tr>
<td><em>N</em>-3′-Maleimidyl)-5-hydroxymethyl-2-pyrrole formaldehyde (166)</td>
<td><em>C. spinosa</em></td>
<td>Fruits</td>
<td>Yang et al., 2010</td>
</tr>
<tr>
<td>Stachydrine (167)</td>
<td><em>C. tomentosa</em></td>
<td>Roots</td>
<td>Akoto et al. (2008)</td>
</tr>
<tr>
<td>Compounds</td>
<td>Source</td>
<td>Plant part</td>
<td>Reference</td>
</tr>
<tr>
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<tr>
<td>Tetrahydroquinoline acid (168)</td>
<td><em>C. spinosa</em></td>
<td>Stems and Fruits</td>
<td>Zhang et al. (2014)</td>
</tr>
<tr>
<td><strong>Long-chain hydrocarbons</strong></td>
<td></td>
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<tr>
<td>Nonadecan-1-ol (169)</td>
<td><em>C. dongvanensis</em></td>
<td>Leaves</td>
<td>Khang et al. (2021)</td>
</tr>
<tr>
<td>Octadecanoic acid (170)</td>
<td><em>C. spinosa</em></td>
<td>Root barks</td>
<td>Khanfar et al. (2003)</td>
</tr>
<tr>
<td>Tetracontane (171)</td>
<td><em>C. dongvanensis</em></td>
<td>Leaves</td>
<td>Khang et al. (2021)</td>
</tr>
<tr>
<td>1-Tetradecanol (172)</td>
<td><em>C. spinosa</em></td>
<td>Root barks</td>
<td>Khanfar et al. (2003)</td>
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<tr>
<td><strong>Flavonoids</strong></td>
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<tr>
<td>Ginkgetin (173)</td>
<td><em>C. spinosa</em></td>
<td>Fruits</td>
<td>H. F. Zhou et al. (2011)</td>
</tr>
<tr>
<td>Isoginkgetin (174)</td>
<td><em>C. spinosa</em></td>
<td>Fruits</td>
<td>H. F. Zhou et al. (2011)</td>
</tr>
<tr>
<td>Isorhamnetin-3-O-rutinoside (175)</td>
<td><em>C. spinosa</em></td>
<td>Root barks</td>
<td>Khanfar et al. (2003)</td>
</tr>
<tr>
<td>Isoquercetin (176)</td>
<td><em>C. sinaica</em></td>
<td>N.I.</td>
<td>Ibrahim et al. (2013)</td>
</tr>
<tr>
<td>Kaempferol (177)</td>
<td><em>C. cartilaginea</em></td>
<td>Leaves</td>
<td>Al-Mahweety and Alyahawi (2020)</td>
</tr>
<tr>
<td></td>
<td><em>C. spinosa</em></td>
<td>Buds</td>
<td>Wiese et al. (2013)</td>
</tr>
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<td></td>
<td><em>C. spinosa</em></td>
<td>Fruits</td>
<td>Zhou et al. (2010)</td>
</tr>
<tr>
<td>Kaempferol-3-O-rutinoside (178)</td>
<td><em>C. spinosa</em></td>
<td>Fruits</td>
<td>H. F. Zhou et al. (2011)</td>
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<tr>
<td>Oroxylin A (179)</td>
<td><em>C. himalayensis</em></td>
<td>Nl.</td>
<td>Li et al. (2008)</td>
</tr>
<tr>
<td>Quercetin (180)</td>
<td><em>C. sinaica</em></td>
<td>Fruits</td>
<td>Zhou et al. (2010)</td>
</tr>
<tr>
<td></td>
<td><em>C. spinosa</em></td>
<td>Buds</td>
<td>Wiese et al. (2013)</td>
</tr>
<tr>
<td>Rutin (181)</td>
<td><em>C. sinaica</em></td>
<td>Fruits</td>
<td>Zhou et al. (2010)</td>
</tr>
<tr>
<td>Sakuranetin (182)</td>
<td><em>C. spinosa</em></td>
<td>Fruits</td>
<td>H. F. Zhou et al. (2011)</td>
</tr>
<tr>
<td>Thevetiaflavone (183)</td>
<td><em>C. spinosa</em></td>
<td>Fruits</td>
<td>Zhou et al. (2010)</td>
</tr>
<tr>
<td>Wogonin (184)</td>
<td><em>C. himalayensis</em></td>
<td>Nl.</td>
<td>Li et al. (2008)</td>
</tr>
</tbody>
</table>

**Glucosinolates and isothiocyanates**

| Glucobrassicin (34)             | *C. spinosa* | Nl.          | Ahmed et al. (1972)    |
| Glucocapparin (185)             | *C. spinosa* | Nl.          | Matthaus and Ozcan (2002) |
| Glucoiberin (186)               | *C. spinosa* | Nl.          | Ahmed et al. (1972)    |
| 3-Methyl-3-buteneisothiocyanate (187) | *C. flexuosa* | Nl.          | Gramosa et al. (1997)  |
| 3-Methyl-2-butenyl-β-glucoside (189) | *C. spinosa* | Root barks   | Khanfar et al. (2003)  |
| Neoglucobrassicin (190)         | *C. spinosa* | Buds         | Wiese et al. (2013)    |

**Phenolic acids**

<p>| Caffeic acid (191)              | <em>C. spinosa</em> | Buds         | Wiese et al. (2013)    |
| 4-Coumaric acid (192)           | <em>C. spinosa</em> | Root barks   | Khanfar et al. (2003)  |
| 3,4-Dihydroxybenzoic acid (193) | <em>C. spinosa</em> | Root barks   | Khanfar et al. (2003)  |</p>
<table>
<thead>
<tr>
<th>Compounds</th>
<th>Source</th>
<th>Plant part</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferulic acid (194)</td>
<td><em>C. spinosa</em></td>
<td>Nl.</td>
<td>Aliyazicioglu et al. (2013)</td>
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<tr>
<td>Gallic acid (195)</td>
<td><em>C. spinosa</em></td>
<td>Buds</td>
<td>Wiese et al. (2013)</td>
</tr>
<tr>
<td>Salicylic acid (196)</td>
<td><em>C. dongvanensis</em></td>
<td>Leaves</td>
<td>Khańg et al. (2021)</td>
</tr>
<tr>
<td>Vanillic acid (197)</td>
<td><em>C. spinosa</em></td>
<td>Fruits</td>
<td>Zhou et al. (2010)</td>
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</tbody>
</table>

**Steroids**

<table>
<thead>
<tr>
<th>Steroids</th>
<th>Source</th>
<th>Plant part</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>5α,6α-Epoxycholestan-3β-ol (198)</td>
<td><em>C. ovata</em></td>
<td>Buds, Fruits, Flowers, Leaves and Stems</td>
<td>Gazioglu et al. (2020)</td>
</tr>
<tr>
<td>5β,6β-Epoxycholestan-3β-ol (199)</td>
<td><em>C. cartilaginea</em></td>
<td>Leaves</td>
<td>Khańg et al. (2021)</td>
</tr>
<tr>
<td>5β,6β-Epoxycholestan-3β-ol (199)</td>
<td><em>C. spinosa</em></td>
<td>Root barks</td>
<td>Khanfar et al. (2003)</td>
</tr>
<tr>
<td>C. ovata</td>
<td></td>
<td>Buds, Fruits, Flowers, Leaves and Stems</td>
<td>Gazioglu et al., 2020</td>
</tr>
<tr>
<td>β-Sitosterol (200)</td>
<td><em>C. decidua</em></td>
<td>Stems</td>
<td>Rathee et al. (2012)</td>
</tr>
<tr>
<td>C. dongvanensis</td>
<td></td>
<td>Leaves</td>
<td>Khańg et al. (2021)</td>
</tr>
<tr>
<td>C. cartilaginea</td>
<td></td>
<td>Leaves</td>
<td>Khańg et al. (2021)</td>
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<tr>
<td>C. spinosa</td>
<td></td>
<td>Root barks</td>
<td>Khanfar et al. (2003)</td>
</tr>
<tr>
<td>C. ovata</td>
<td></td>
<td>Buds, Fruits, Flowers, Leaves and Stems</td>
<td>Gazioglu et al., 2020</td>
</tr>
</tbody>
</table>

**β-Sitosterol 3-O-β-D-glucopyranoside (201)**

<table>
<thead>
<tr>
<th>β-Sitosterol 3-O-β-D-glucopyranoside (201)</th>
<th>Source</th>
<th>Plant part</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. dongvanensis</em></td>
<td></td>
<td>Leaves</td>
<td>Khańg et al. (2021)</td>
</tr>
<tr>
<td>Compounds</td>
<td>Source</td>
<td>Plant part</td>
<td>Reference</td>
</tr>
<tr>
<td>-----------</td>
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</tr>
<tr>
<td>C. spinosa</td>
<td>Root barks</td>
<td>Khanfar et al. (2003)</td>
<td></td>
</tr>
</tbody>
</table>

**Terpenoids**

<p>| Capparosol A (203) | C. spinosa | Root barks | Khanfar et al. (2003) |
| Dihydroxy-lup-20(29)-en-28-oic acid (204) | C. cartilaginea | Leaves | Al-Mahweety and Alyahawi (2020) |
| (+)-(65,95)-9-O-β-D-Glucopyranosyloxy-6-hydroxy-3oxo-α-ionol (205) | C. spinosa | Root barks | Khanfar et al. (2003) |
| (−)-(65,95)-9-O-β-D-Glucopyranosyloxy-6,13-dihydroxy-3-oxo-α-ionol (206) | M. siamensis | Leaves and Twigs | Nobsathian et al. (2018) |
| Lupeol (207) | M. siamensis | Leaves and Twigs | Nobsathian et al. (2018) |
| Olean-12-en-3β,28-diol 3β-pentacosanoate (208) | C. ovata | Buds, Fruits, Flowers, Leaves and Stems | Gazioglu et al. (2020) |
| Oleanolic acid (209) | C. ovata | Buds, Fruits, Flowers, Leaves and Stems | Gazioglu et al. (2020) |
| Simiarenol (210) | C. spinosa | Root barks | Khanfar et al. (2003) |
| Taraxerol (211) | C. ovata | Buds, Fruits, Flowers, Leaves and Stems | Gazioglu et al. (2020) |</p>
<table>
<thead>
<tr>
<th>Compounds</th>
<th>Source</th>
<th>Plant part</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ursolic acid (212)</td>
<td>C. spinosa</td>
<td>Root barks</td>
<td>Khanfar et al. (2003)</td>
</tr>
<tr>
<td><strong>Miscellaneous</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Benzoic acid (213)</td>
<td>C. dongvanensis</td>
<td>Leaves</td>
<td>Calis et al. (1999)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Khanh et al. (2021)</td>
</tr>
<tr>
<td>Bismethyl-octylphthalate (214)</td>
<td>C. ovata</td>
<td>Buds, Fruits, Flowers,</td>
<td>Gazioglu et al. (2020)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Leaves and Stems</td>
<td></td>
</tr>
<tr>
<td>(+)-R-2-(4-Hydroxy-2-oxo-2,3-dihydrobenzofuran-3-yl) acetonitrile (216)</td>
<td>C. spinosa</td>
<td>Fruits and Stems</td>
<td>Zhang et al. (2014)</td>
</tr>
<tr>
<td>(+)-S-2-(4-Hydroxy-2-oxo-2,3-dihydrobenzofuran-3-yl) acetonitrile (217)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Nicotinamide (218)</td>
<td>C. spinosa</td>
<td>Root barks</td>
<td>Khanfar et al. (2003)</td>
</tr>
<tr>
<td><em>Para</em>-hydroxybenzaldehyde (219)</td>
<td>C. spinosa</td>
<td>Root barks</td>
<td>Khanfar et al. (2003)</td>
</tr>
<tr>
<td>Phthalic acid (220)</td>
<td>C. decidua</td>
<td>Root barks</td>
<td>Forster et al. (2016)</td>
</tr>
<tr>
<td>Tryptophan (221)</td>
<td>C. dongvanensis</td>
<td>Leaves</td>
<td>Khanh et al. (2021)</td>
</tr>
</tbody>
</table>

Nl. refers to “not indicated”.
Alkaloids

14-N-Acetylcodonocarpine (146); $R_1 = \text{OCH}_3$, $R_2 = \text{OH}$
15-N-Acetylcodonocarpine (147); $R_1 = \text{OH}$, $R_2 = \text{OCH}_3$

Cadabicine; $R_1 = R_2 = \text{H}$ (149)
Capparidisine; $R_1 = R_2 = \text{OCH}_3$ (150)
Capparidisine; $R_1 = \text{OCH}_3$, $R_2 = \text{OH}$, $R_3 = \text{OCH}_3$, $R_4 = \text{OCH}_3$ (151)

Capparidine; $R_1 = \text{OH}$, $R_2 = \text{H}$, $R_3 = \text{OCH}_3$, $R_4 = \text{H}$ (156)

Codonocarpine; $R_1 = \text{OH}$, $R_2 = \text{OCH}_3$, $R_3 = \text{H}$, $R_4 = \text{H}$ (161)

Cappariloside A (152)  Cappariloside B (153)

Capparin A (154)  Capparine B (155); $R_1 = \text{H}$, $R_2 = \text{OCH}_3$, $R_3 = \text{SCH}_3$

4-Hydroxy-1H-indole-3-carbaldehyde (163); $R_1 = \text{OH}$, $R_2 = R_3 = \text{H}$
3-Hydroxy-3-methyl-4-methoxoxindole (165)

N-(3'-Maleimidyl)-5-hydroxymethyl-2-pyrrrole formaldehyde (166)

Stachydrine; R= H (167)

Tetrahydroquinoline acid (168)

Long chain hydrocarbons

Nonadecan-1-ol (169)

Octadecanoic acid (170)

Tetracontane (171)
Flavonoids

Ginkgetin (173); R₁ = CH₃, R₂ = H
Isoginkgetin (174); R₁ = H, R₂ = CH₃

isorhamnetin-3-O-rutinoside (175); R₁ = rutinoside, R₂ = OCH₃
Isoquercetin (176); R₁ = Glu, R₂ = OH
Kaempferol (177); R₁ = R₂ = H
Kaempferol-3-O-rutinoside (178); R₁ = rutinoside, R₂ = H
Quercetin (180); R₁ = H, R₂ = OH
Rutin (181); R₁ = rutinoside, R₂ = OH

Oroxylin A (179); R₁ = OCH₃, R₂ = H
Wogonin (184); R₁ = H, R₂ = OCH₃

Sakuranetin (182)

1-Tetradecanol (172)
Glucosinolates and isothiocyanates

Thevetiaflavone (183)

Glucocapparin (185)  Glucoiberin (186)

3-Methyl-3-butenoisothiocyanate (187)  3-Methyl-2-butenyl-β-glucoside (188)
Phenolic acids

Caffeic acid \((\text{191})\); \(R = \text{OH}\)
4-Coumaric acid \((\text{192})\); \(R = \text{H}\)
Ferulic acid \((\text{194})\); \(R = \text{OCH}_3\)
3,4-Dihydroxybenzoic acid \((\text{193})\); \(R_1 = R_2 = \text{OH, } R_3 = \text{H}\)
Salicylic acid \((\text{196})\); \(R_1 = R_2 = \text{H, } R_3 = \text{OH}\)

Steroids

\(5\alpha,6\alpha\)-Epoxysterol-3\(\beta\)-ol \((\text{198})\)
\(5\beta,6\beta\)-Epoxysterol-3\(\beta\)-ol \((\text{199})\)
**Terpenoids**

- **β-Sitosterol** (200); R= OH
- **β-Sitosterol 3-O-β-D-glucopyranoside** (201); R= O-Glu
- **Stigmasterol** (202)

Capparisol A (203)

Dihydroxy-lup-20(29)-en-28-oic acid (204)

(+)-(6S,9S)-9-O-β-D-Glucopyranosyloxy-6-hydroxy-3-oxo-α-ionol (205)
(-)-(6S,9S)-9-O-\(\beta\)-D-Glucopyranosyloxy-6,13-dihydroxy-3-oxo-\(\alpha\)-ionol (206)

Lupeol (207) Olean-12-en-3\(\beta\),28-diol 3\(\beta\)-pentacosanoate (208)

Oleanolic acid (209) Simiarenol (210)
Taraxerol (211)

Ursolic acid (212)

Benzoic acid (213)

Bismethyl-octylphthalate (214)

Guanosine (215)
(+)-R-2-(4-Hydroxy-2-oxo-2,3-dihydrobenzofuran-3-yl) acetonitrile (216)

(+)-S-2-(4-Hydroxy-2-oxo-2,3-dihydrobenzofuran-3-yl) acetonitrile (217)

Nicotinamide (218)  
para-Hydroxybenzaldehyde (219)

Phthalic acid (220)  
Tryptophan (221)
Table 3. Chemical constituents of plants in the genus *Maerua*

<table>
<thead>
<tr>
<th>Compound</th>
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<tr>
<td><strong>Alkaloids</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Cappariloside A (152)</td>
<td><em>M. siamensis</em></td>
<td>Leaves and Twigs</td>
<td>Nobsathian <em>et al.</em> (2018)</td>
</tr>
<tr>
<td>Cappariloside B (153)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>cis</em>-Cinnamoyl-4-aminobutylguanidine (222)</td>
<td><em>M. edulis</em></td>
<td>Leaves</td>
<td>Stevenson <em>et al.</em> (2018)</td>
</tr>
<tr>
<td><em>trans</em>-Cinnamoyl-4-aminobutylguanidine (223)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>4-Hydroxy-<em>E</em>-cinnamoyl-4-aminobutylguanidine (224)</td>
<td></td>
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<tr>
<td>4-Hydroxy-<em>Z</em>-cinnamoyl-4-aminobutylguanidine (225)</td>
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<tr>
<td>Stachydrine (167)</td>
<td><em>M. crassifolia</em></td>
<td>Aerial parts</td>
<td>Bishay <em>et al.</em> (1990)</td>
</tr>
<tr>
<td></td>
<td><em>M. edulis</em></td>
<td>Leaves</td>
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<tr>
<td><strong>Flavonoids</strong></td>
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</tr>
<tr>
<td>Chrysoeriol (226)</td>
<td><em>M. siamensis</em></td>
<td>Leaves and Twigs</td>
<td>Nobsathian <em>et al.</em> (2018)</td>
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<tr>
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<tr>
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<td></td>
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</tr>
<tr>
<td>Quercetin (180)</td>
<td><em>M. crassifolia</em></td>
<td>Aerial parts</td>
<td>Ibraheim (1995)</td>
</tr>
<tr>
<td>Quercetin-3-<em>O</em>-arabinopyranoside (228)</td>
<td><em>M. crassifolia</em></td>
<td>Aerial parts</td>
<td>Bishay <em>et al.</em> (1990)</td>
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<td>Quercetin-3-<em>O</em>-β-D-galactoside (229)</td>
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<tr>
<td><strong>Lignan</strong></td>
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</tr>
<tr>
<td>Lyoniresinol-3-<em>O</em>-glucopyranoside (230)</td>
<td><em>M. crassifolia</em></td>
<td>Aerial parts</td>
<td>Bishay <em>et al.</em> (1990)</td>
</tr>
<tr>
<td><strong>Steroids</strong></td>
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<tr>
<td>Compound</td>
<td>Source</td>
<td>Plant part</td>
<td>Reference</td>
</tr>
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<td>--------------</td>
<td>------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>Lupeol (207)</td>
<td><em>M. siamensis</em></td>
<td>Leaves and Twigs</td>
<td>Nobsathian et al. (2018)</td>
</tr>
<tr>
<td><strong>Terpenoids</strong></td>
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</tr>
<tr>
<td>Betulin (231)</td>
<td><em>M. oblongifolia</em></td>
<td>Aerial parts</td>
<td>Abdel-Moğib (1999)</td>
</tr>
<tr>
<td>Betulinaldehyde (232)</td>
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<td></td>
</tr>
<tr>
<td>Glochidone (233)</td>
<td><em>M. siamensis</em></td>
<td>Leaves and Twigs</td>
<td>Nobsathian et al. (2018)</td>
</tr>
<tr>
<td>Hexahydrofarnesyl acetone (234)</td>
<td><em>M. oblongifolia</em></td>
<td>Aerial parts</td>
<td>Abdel-Moğib (1999)</td>
</tr>
<tr>
<td>Ionol glucoside (235)</td>
<td><em>M. crassifolia</em></td>
<td>Aerial parts</td>
<td>Ibraheim (1995)</td>
</tr>
<tr>
<td>Lup-20(29)-en-3β,30-diol (236)</td>
<td><em>M. oblongifolia</em></td>
<td>Aerial parts</td>
<td>Abdel-Moğib (1999)</td>
</tr>
<tr>
<td>Phytol (237)</td>
<td><em>M. oblongifolia</em></td>
<td>Aerial parts</td>
<td>Abdel-Moğib (1999)</td>
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<tr>
<td><strong>Miscellaneous</strong></td>
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<tr>
<td>Cinnamic acid (238)</td>
<td><em>M. siamensis</em></td>
<td>Leaves and Twigs</td>
<td>Nobsathian et al. (2018)</td>
</tr>
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<td>3,4-Dihydroxybenzoic acid (193)</td>
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<tr>
<td>Guaiacyl glycerol (239)</td>
<td><em>M. crassifolia</em></td>
<td>Aerial parts</td>
<td>Ramadan et al. (1999)</td>
</tr>
<tr>
<td>6-N-Methyl-9-β-D-glucoside adenine (240)</td>
<td><em>M. crassifolia</em></td>
<td>Aerial parts</td>
<td>Ramadan et al. (1999)</td>
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<tr>
<td>3,4,5-Trimethoxyphenol-1-O-β-D-glucopyranoside (241)</td>
<td><em>M. siamensis</em></td>
<td>Leaves and Twigs</td>
<td>Nobsathian et al. (2018)</td>
</tr>
<tr>
<td>Vanillin (242)</td>
<td><em>M. siamensis</em></td>
<td>Leaves and Twigs</td>
<td>Nobsathian et al. (2018)</td>
</tr>
</tbody>
</table>
Alkaloids

cis-Cinnamoyl-4-aminobutylguanidine (222); R = H
4-Hydroxy-Z-cinnamoyl-4-aminobutylguanidine (225); R = OH

Flavonoids

Chrysoeriol (226); R₁ = H, R₂ = OCH₃
Kaempferol-3-O-galactorhamnoside (227); R₁ = galactose–rhamnose, R₂ = H
Quercetin-3-O-arabinopyranoside (228); R₁ = arabinose, R₂ = OH
Quercetin-3-O-β-D-galactoside (229); R₁ = β-D-galactose, R₂ = OH
**Lignans**

Lyoniresinol-3-O-glucopyranoside (230)

**Terpenoids**

Betulin (231); \( R_1 = CH_3OH, R_2 = CH_3 \)

Betulinaldehyde (232); \( R_1 = CHO, R_2 = CH_3 \)

Lup-20(29)-en-3\textbeta;30-diol (236); \( R_1 = CH_3, R_2 = CH_3OH \)

Hexahydrofarnesyl acetone (234)
Ionoglucoside (235)

Phytol (237)

Cinnamic acid (238)

Guaiacyl glycerol (239)

6-N-Methyl-9-β-D-glucoside adenine (240)

3,4,5-Trimethoxyphenol-1-O-β-D-glucopyranoside (241)

Vanillin (242)
CHAPTER III
EXPERIMENTAL

3.1 Source of plant materials

The stems of Capparis micracantha were collected from Saraburi province in March 2019, while the roots of Maerua siamensis were collected from Sikhio district, Nakhon Ratchasima in April 2019. Voucher specimens of these plants have been deposited at department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand.

3.2 General techniques

3.2.1 Solvents

Organic solvents used in this study were commercial grade and were distilled before used.

3.2.2 Analytical normal-phase thin-layer chromatography (TLC)

<table>
<thead>
<tr>
<th>Technique</th>
<th>One dimensional, ascending</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbent</td>
<td>Silica gel 60 F254 (Merck, Darmstadt, Germany)</td>
</tr>
<tr>
<td>Layer thickness</td>
<td>0.2 mm</td>
</tr>
<tr>
<td>Distance</td>
<td>5 cm</td>
</tr>
<tr>
<td>Temperature</td>
<td>Room temperature (30-32 °C)</td>
</tr>
<tr>
<td>Detection</td>
<td>1. Ultraviolet light (wavelengths of 254 and 365 nm)</td>
</tr>
<tr>
<td></td>
<td>2. Spraying with Ce₂(SO₄)₃ reagent and heating at 110-130 °C for 5 min</td>
</tr>
</tbody>
</table>

3.2.3 Analytical reversed-phase thin-layer chromatography (RP-18)

<table>
<thead>
<tr>
<th>Technique</th>
<th>One dimensional ascending</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbent</td>
<td>Silica gel 60 RP-18 F₂₅₄S (Merck) No. 1.05559</td>
</tr>
<tr>
<td>Layer thickness</td>
<td>0.2 mm</td>
</tr>
<tr>
<td>Distance</td>
<td>5 cm</td>
</tr>
<tr>
<td>Temperature</td>
<td>Room temperature (30-32 °C)</td>
</tr>
</tbody>
</table>
Detection: as described in section 3.2.2

3.2.4 Column chromatography

3.2.4.1 Flash Column Chromatography

Adsorbent: Silica gel 60, 230–400 mesh (Merck)

Packing method: Dry packing

Sample loading: The sample was dissolved in a small amount of organic solvent. Then, a small quantity of silica was mixed with the sample and dried. After that, the mixture was placed on top of the column.

Detection: Fractions were examined as described in section 3.2.2.

3.2.4.2 Medium Performance Liquid Column Chromatography (MPLC)

Adsorbent: Silica gel 60 (70–230 or 230–400 mesh) and LiChroprep® RP-18 (25–40 μm) (Merck)

Packing method: Dry packing

Sample loading: The sample was dissolved in a small amount of organic solvent. Then, a small quantity of silica was mixed with the sample and dried. After that, the mixture was placed on top of the column.

Detection: Fractions were examined as described in section 3.2.2.

3.2.4.3 Conventional column chromatography

Adsorbent: Silica gel 60 (70–230 or 230–400 mesh) (Merck)

Packing method: Dry packing

Sample loading: The sample was dissolved in a small amount of organic solvent. Then, a small quantity of silica was mixed with the sample and dried. After that, the mixture was placed on top of the column.
Detection: Fractions were examined as described in section 3.2.2.

3.2.4.4 Gel filtration chromatography

Adsorbent: Sephadex LH-20 (GE Healthcare, Amersham, UK)

Packing method: Sephadex gel was allowed to swell in mobile phase for 24 hours, then poured into the column.

Sample loading: The sample was dissolved in a small amount of mobile phase, then placed on top of the column.

Detection: Fractions were examined as described in section 3.2.2.

3.3 Spectroscopy

3.3.1 Ultraviolet absorption spectra

Ultraviolet (UV) spectra were obtained on a Milton Roy Spectronic 3000 Array spectrophotometer (Rochester, NY, USA) at Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

3.3.2 Infrared spectra

Fourier Transform Infrared (FT-IR) spectra was recorded on a Thermo scientific Nicolet™ iS50 FT-IR spectrometer (Thermo Fisher scientific, Waltham, MA, USA) at the National Nanotechnology Center (NANOTEC, Thailand) or a Perkin Elmer FT-IR 1760X spectrometer (Boston, MA, USA) spectrometer (Scientific and Technological Equipment Center, Chulalongkorn University).

3.3.3 Mass spectrometer

High Resolution-Electron Spray Ionization-Mass Spectrometry (HR-ESI-MS) spectra were obtained on a Bruker APEX II mass spectrometer (Karlsruhe, Germany) at Kaohsiung Medical University (Taiwan), or an Agilent 6540 UHD Accurate-Mass Q-TOF mass spectrometer (CA, USA) at the Science Lab Center, Faculty of Science, Naresuan University.
3.3.4 Proton and carbon-13 nuclear magnetic resonance (\(^1\)H- and \(^{13}\)C-NMR) spectroscopy

\(^1\)H-NMR (600 MHz) and \(^{13}\)C-NMR (150 MHz) spectra were recorded on a Varian VNMRS-600 spectrometer (Lexington, MA, USA) Kaohsiung Medical University and \(^1\)H NMR (400 MHz) and \(^{13}\)C NMR (100 MHz) spectra were recorded on a Bruker Advance NEO 400 MHz NMR spectrometer (Karlsruhe, Germany) at the Faculty of Pharmaceutical Sciences, Chulalongkorn University.

3.3.5 Circular dichroism

Circular dichroism was measured on a JASCO J-815CD/ORD spectropolarimeter (Kyoto, Japan) at Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

3.3.6 Polarimetry

3.3.6.1 Optical rotation

Specific rotation values were measured using a JASCO P-2000 polarimeter (Kyoto, Japan) at Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

3.4. Extraction and isolation

3.4.1 Extraction of Capparis micracantha stems

Dried stems of C. micracantha (3.0 kg) were cut into small pieces and macerated with methanol (MeOH) 3×8 L, for three days each. The methanol extract was evaporated under reduced pressure to obtain crude MeOH extract (350 g, 11.67 % yield, based on dried weight of stems). The extract was redissolved in MeOH added with deionized (DI) water, then partitioned with hexane (6 L), ethyl acetate (EtOAc, 8 L), and n-butanol (8 L) successively to give hexane extract (13.8 g, 0.46% yield), EtOAc extract (11.5 g, 0.38% yield), n-butanol extract (56.5 g, 1.9 % yield) and aqueous extract (265.3 g, 8.84 % yield). (Scheme 1.)
3.4.2 Isolation of compounds from the EtOAc extract of *Capparis micracantha* stems (CMSE)

The EtOAc extract (11.45 g) was divided into 4 portions and each portion was separated by MPLC using silica gel as stationary phase. The column was eluted with *n*-hexane-acetone (4:1 to 0:1) and washed by CH$_2$Cl$_2$-MeOH (1:1). Each fraction was collected about 50 mL of eluate. All fractions were combined according to their TLC pattern to give 8 major fractions: CMSE-1 (0.7 g), CMSE-2 (1.2 g), CMSE-3 (1.8 g), CMSE-4 (1.6 g), CMSE-5 (2.7 g), CMSE-6 (2.4 g), CMSE-7 (1.5 g), and CMSE-8 (2.5 g).
3.4.2.1 Isolation of compound 1 (methyl 6-methoxy-3-indolecarbonate)

Fraction CMSE-4 was combined with CMSE-5 and separated by MPLC using silica gel as stationary phase. The mobile phase was n-hexane-acetone (4:1 to 0:1). The eluates were combined into 8 fractions (Fr.4-1 to 4-8). Fraction 4-3 (99.8 mg) was purified by size exclusion chromatography (Sephadex LH-20), eluted with CH₂Cl₂-MeOH (1:1) to give 4 subfractions (fr.4-3-1 — 4-3-4). Subfraction 4-3-4 (10 mg) was further repurified by Sephadex LH-20 column (MeOH) to obtain 10 subfractions. **Compound 1** (1.2 mg) was obtained from subfraction 4-3-4-5 as a yellow amorphous solid (Scheme 2).

3.4.2.2 Isolation of compound 2 (vanillic acid)

Fraction 4-5 (147.2 mg) was subjected to silica gel column chromatography (Si-CC), eluted with CH₂Cl₂-acetone (30:1 to 0:1) to gain 8 subfractions (fr.4-5-1 — 4-5-8). Subfraction 4-5-2 (49 mg) was further separated by Si-CC with n-hexane-EtOAc (3:2) as the mobile phase to afford 6 subfractions (fr.4-5-2-1 — 4-5-2-6). Subfraction 4-5-2-3 was further purified by recrystallization in CH₂Cl₂—MeOH to obtain **compound 2** (4.1 mg) as a white amorphous solid (Scheme 2).

3.4.2.3 Isolation of compound 3 [(−)-syringaresinol]

Fraction 4-6 (537.8 mg) was separated by MPLC using silica gel as stationary phase and CH₂Cl₂-acetone (10:1) as mobile phase to yield 7 subfractions (Fr.4-6-1 — 4-6-7). Subfraction 4-6-2 (81.3 mg) was subjected to Si-CC using CH₂Cl₂—MeOH (20:1) as the solvent system to obtain 13 subfractions (fr.4-6-2-1 — 4-6-2-13). Subfraction 4-6-2-4 (38.5 mg) was further separated on a Sephadex LH-20 column (MeOH) to obtain 6 subfractions (fr.4-6-2-4-1 — 4-6-2-4-6). Subfraction 4-6-2-4-3 yielded **compound 3** (10.0 mg) as a white amorphous solid (Scheme 2).
3.4.2.4 Isolation of compound 4 [(+)-ampelopsin A]

Fraction CMSE 6 (2.4 g) was subjected to Sephadex LH-20 column eluted with MeOH to give 8 fractions (fr.6-1 – 6-8). Fraction 6-4 (32.5 mg) was repurified on Sephadex LH-20 column (MeOH) to yield 6 subfractions (fr.6-4-1 – 6-4-6). Subfraction 6-4-4 (32.8 mg) was further separated by MPLC using reverse phase (RP-18) column as stationary phase. After eluting with DI water—MeOH (3:2 to 1:1), 8 subfractions (fr.6-4-4-1 – 6-4-4-8) were afforded. Compound 4 (2.4 mg) was purified from subfraction 6-4-4-4 (Scheme 3).

3.4.2.5 Isolation of compound 5 [(-)-pauciflorol E]

Fraction 6-6 (162.0 mg) was loaded on MPLC [silica gel, CH₂Cl₂—acetone (4:1 to 0:1)] to gain 12 subfractions. Subfractions 6-6-7 (7.9 mg) was purified by Si-CC using CH₂Cl₂—MeOH (20:1) to obtain 4 subfractions (fr.6-6-7-1 – 6-6-7-4). Subfractions 6-6-7-1 (5.1 mg) was further purified by Sephadex LH-20 (MeOH) to give 4 subfractions. Compound 5 (3.0 mg) was obtained from subfraction 6-6-7-1-3 as a green amorphous solid (Scheme 3).
**Scheme 2.** Isolation of compounds 1 – 3 from the EtOAc extract of *C. micracantha*

EtOAc extract (11.45 g) → silica gel, n-hexane-acetone (4:1 to 0:1)

fr.1-3 → fr.4-6
→ silica gel, n-hexane-acetone (4:1 to 0:1)
→ fr.4-6 → fr.4-6-1
→ silica gel, CH₂Cl₂–acetone (30:1 to 0:1)
→ fr.4-6-1 → fr.4-6-2
→ silica gel, CH₂Cl₂–acetone (10:1)
→ fr.4-6-2 → fr.4-6-3 to 7
→ silica gel, n-hexane–CH₂Cl₂ (3:1)
→ fr.4-6-3 to 7 → fr.4-6-2-1 to 3
→ silica gel, n-hexane–CH₂Cl₂ (20:1)
→ fr.4-6-2-1 to 3 → fr.4-6-5-2
→ silica gel, n-hexane–EtOAc (3:1)
→ fr.4-6-5-2 → fr.4-5-2-3
→ silica gel, n-hexane–acetone (30:1 to 0:1)
→ fr.4-5-2-3 → fr.4-5-2-4 to 4
→ silica gel, n-hexane–acetone (10:1)
→ fr.4-5-2-4 to 4 → fr.4-5-2-5 to 13
→ silica gel, n-hexane–acetone (20:1)
→ fr.4-6-5-2 → fr.4-6-2-2
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-2 → fr.4-6-2-4
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-4 → fr.4-6-2-5 to 13
→ silica gel, n-hexane–acetone (10:1)
→ fr.4-6-2-5 to 13 → fr.4-6-2-6
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-6 → fr.4-6-2-7
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-7 → fr.4-6-2-8
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-8 → fr.4-6-2-9
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-9 → fr.4-6-2-10
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-10 → fr.4-6-2-11
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-11 → fr.4-6-2-12
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-12 → fr.4-6-2-13
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-13 → fr.4-6-2-14
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-14 → fr.4-6-2-15
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-15 → fr.4-6-2-16
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-16 → fr.4-6-2-17
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-17 → fr.4-6-2-18
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-18 → fr.4-6-2-19
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-19 → fr.4-6-2-20
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-20 → fr.4-6-2-21
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-21 → fr.4-6-2-22
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-22 → fr.4-6-2-23
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-23 → fr.4-6-2-24
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-24 → fr.4-6-2-25
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-25 → fr.4-6-2-26
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-26 → fr.4-6-2-27
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-27 → fr.4-6-2-28
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-28 → fr.4-6-2-29
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-29 → fr.4-6-2-30
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-30 → fr.4-6-2-31
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-31 → fr.4-6-2-32
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-32 → fr.4-6-2-33
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-33 → fr.4-6-2-34
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-34 → fr.4-6-2-35
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-35 → fr.4-6-2-36
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-36 → fr.4-6-2-37
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-37 → fr.4-6-2-38
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-38 → fr.4-6-2-39
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-39 → fr.4-6-2-40
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-40 → fr.4-6-2-41
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-41 → fr.4-6-2-42
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-42 → fr.4-6-2-43
→ silica gel, n-hexane–acetone (4:1 to 0:1)
→ fr.4-6-2-43 (10 mg)
→ Compound 3

---

**Scheme 2.** Isolation of compounds 1 – 3 from the EtOAc extract of *C. micracantha*
Scheme 3. Isolation of compounds 4 — 5 from the EtOAc extract of *C. micrantha*
3.4.3 Extraction of *Maerua siamensis* roots

Dried roots of *M. siamensis* (7.0 kg) were cut into small pieces and extracted with EtOAc (3×30 L), for three days each. The extract was evaporated under vacuum to yield EtOAc extract (29.3 g, 0.42 % yield). The marc was further macerated with MeOH (3× 30 L), for three days each. After evaporation, the MeOH extract (350 g, 5.0 % yield) was redissolved in MeOH, added with DI water and partitioned with n-butanol (8 L) to give n-butanol extract (50.8 g, 0.73 % yield) and aqueous extracts (299.2 g, 4.27 % yield). (Scheme 4.)

![Scheme 4. Extraction of Maerua siamensis roots](image-url)
3.4.4 Isolation of compounds from EtOAC extract of *Maerua siamensis* roots

The EtOAc extract (29.3 g) was separated by a silica gel MPLC column using *n*-hexane-acetone (15:1 to 6:1) as mobile phase. The flow rate was 15 mL/min. Each collected fraction was 50 mL. Based on TLC pattern, 9 major fractions were obtained as follows: MSRE-1 (5.30 g), MSRE-2 (4.17 g), MSRE-3 (1.10 g), MSRE-4 (0.20 g), MSRE-5 (0.18 g), MSRE-6 (0.27 g), MSRE-7 (0.25 g), MSRE-8 (2.14 g), and MSRE-9 (1.06 g).

3.4.4.1 Isolation of compound 6 [(+)-maeruanitrile A] and compound 7 (maeruanitrile B)

Fraction MRSE-8 (2.14 g) was subjected to MPLC [silica gel, CH$_2$Cl$_2$—acetone (120:1 to 20:1)] to give 12 fractions (fr.8-1 — 8-12). Fraction 8-2 (66.6 mg) was separated using MPLC [silica gel, *n*-hexane—CH$_2$Cl$_2$—acetone (8:1:1 to 4:1:1)] to gain 12 subfractions (fr.8-2-1 — 8-2-12). Subfraction 8-2-4 (28.60 mg) was purified in two steps using *n*-hexane—CH$_2$Cl$_2$—acetone system [8:1:1 to 4:1:1 and 4:1:1 to 2:1:1] to yield 14 subfractions (fr.8-2-4B-1—8-2-4B-14). Subfraction 8-2-4B-14 was subjected to Si-CC eluted with *n*-hexane—CH$_2$Cl$_2$—acetone (2:1:1) to afford compound 6 (2.4 mg) as a reddish-brown amorphous solid from subfraction 8-2-4B-14, and compound 7 (1.4 mg) as a reddish-brown amorphous solid from subfraction 8-2-4B-14-8 (Scheme 5).

3.4.4.2 Isolation of compound 8 (maeroxime A)

Fraction MRSE-6 (274.50 mg) was loaded on MPLC [silica gel, *n*-hexane-acetone (3:1)], to yield 10 fractions (fr.6-1 — 6-10). Fraction 6-5 (38.2 mg) was further separated by MPLC using C-18 reversed phase silica gel as stationary phase and DI water—acetonitrile (1:2) as mobile phase to gain 13 subfractions (Fr.6-5-1 — 6-5-13). Subfraction 6-5-2 (8 mg) was purified by preparative reversed phase TLC using DI water—acetonitrile (1:4) as mobile phase to afford compound 8 (2.4 mg) as a reddish-brown amorphous solid from fraction 6-5-2-2 (Scheme 6).
3.4.4.3 Isolation of compound 9 (maeroxime B)

Fraction MRSE-8 (2.14 g) was subjected to MPLC [silica gel, CH$_2$Cl$_2$—acetone (120:1 to 20:1)] to give 12 fractions (fr.8-1 — 8-12). Fraction 8-6 (31.90 mg) was undergone three-step purification by Si-CC using with n-hexane—acetone (10:1 to 6:1) and n-hexane—acetone (6:1), and n-hexane—acetone (3:1) to obtain compound 9 (fraction 8-6-13-3A, 1.0 mg) as an orange-brown amorphous solid (Scheme 7).

3.4.4.4 Isolation of compound 10 (maeroxime C)

Fraction 8-9 (158 mg) was subjected to three-step purification by MPLC [Si-CC, n-hexane—CH$_2$Cl$_2$—acetone (8:1:1, 8:1:1 and 6:1:1)] to obtain subfraction 8-9B-11-7. Subfraction 8-9B-11-7 was further separated by a Sephadex LH-20 column (MeOH) to give 8 subfractions (fr.8-9B-11-7-1 — 8-9B-11-7-8). Compound 10 (3.5 mg) was obtained from subfraction 8-9B-11-7-4 as a yellow amorphous solid (Scheme 8).
Scheme 5. Isolation of compound 6 – 7 from the EtOAc extract of *M. siamensis*
Scheme 6. Isolation of compound 8 from the EtOAc extract of *M. siamensis*
Scheme 7. Isolation of compound 9 from the EtOAc extract of *M. siamensis*
Scheme 8. Isolation of compound 10 from the EtOAc extract of *M. siamensis*
3.4.5 Isolation of compounds from \textit{n}-butanol extract of \textit{M. siamensis} roots

The \textit{n}-butanol extract (50.8 g) was divided into 4 portions and loaded on Sephadex LH-20 (MeOH). All fractions were combined to yield 5 major fractions—MSRB-A (26.5 g), MSRB-B (20.8 g), MSRB-C (5.7 g), MSRB-D (5.7 g) and MSRB-E (5.2 g).

3.4.5.1 Isolation of compounds 11 (maeruabisindole A)

Fraction MSRB-C (5.7 g) was done with a separation on MPLC [silica gel, CH$_2$Cl$_2$-acetone (10:1 to 1:1)] to gain 16 subfractions (fr.C-1 \textendash{} C-16). Subfraction MSRB-C-7 was further purified using MPLC [silica gel, CH$_2$Cl$_2$-acetone (10:1)] to obtain 10 subfractions (fr.C-7-1 \textendash{} C-7-10). Subfraction C-7-5 (283.5 mg) was separated into two steps by Sephadex LH-20 (MeOH) to yield compound 11 (subfraction MSRB-C-7-5-9-4, 1.1 mg) as a pale green amorphous solid (Scheme 9).

3.4.5.2 Isolation of compounds 12 (maeruabisindole B)

Fraction MSRB-C-15 (3.58 g) was separated by MPLC [Si-CC, CH$_2$Cl$_2$-acetone (6:1)] to yield 11 subfractions (fr.C-15-1 \textendash{} C-15-11). Fraction C-15-4 (8.6 mg) was further purified by Sephadex LH-20 (MeOH) to obtain 4 subfractions (fr.C-15-4-1 \textendash{} C-15-4-4). Compound 12 (1.1 mg) was yielded as a pale green amorphous solid from subfraction MSRB-C-15-4-1 (Scheme 10).

3.4.5.3 Isolation of compounds 13 (maeruabisindole C)

Fraction MSRB-E (2.31 g) was separated by MPLC column [silica gel, CH$_2$Cl$_2$-acetone (10:1 to 1:1)] to obtain 12 subfractions (fr.E-1 \textendash{} E-12). Compound 13 (3.5 mg) was obtained as a dark green amorphous solid from subfraction MSRB-E-4 (Scheme 11).
Scheme 9. Isolation of compound 11 from the butanol extract of *M. siamensis*
Scheme 10. Isolation of compounds 12 from the butanol extract of M. siamensis
Scheme 11. Isolation of compound 13 from the butanol extract of *M. siamensis*.
3.5 Physical and spectral data of isolated compounds

3.5.1 Compound 1 (methyl 6-methoxy-3-indolecarbonate)

**Compound 1** was obtained as a yellow amorphous solid, soluble in acetone (1.2 mg, 0.00004% based on dried weight of stems).

HR-ESI-MS: [M+H]$^+$ at m/z 206.0817 (calculated for C$_{11}$H$_{12}$NO$_3$, 206.0818); Figure 5

FT-IR: $\nu_{\text{max}}$ (ATR) cm$^{-1}$: 3307, 2947, 2836, 1678, 1532, 1442, 1277, 1153; Figure 6

$^1$H-NMR: $\delta$ ppm, 400 MHz, acetone-$d_6$; see Table 4; Figure 7-8

$^{13}$C-NMR: $\delta$ ppm, 100 MHz, acetone-$d_6$; see Table 4; Figure 9

3.5.2 Compound 2 (vanillic acid)

**Compound 2** was obtained as a white amorphous solid, soluble in acetone (4.1 mg, 0.00014% based on dried weight of stems).

HR-ESI-MS: [M+H]$^+$ at m/z 169.0504 (calculated for C$_8$H$_9$O$_4$, 169.0501); Figure 15

FT-IR: $\nu_{\text{max}}$ (ATR) cm$^{-1}$: 3484, 2923, 1674, 1595, 1522, 1433, 1217, 1237, 1202, 1111, 763, 503; Figure 16

$^1$H-NMR: $\delta$ ppm, 400 MHz, acetone-$d_6$; see Table 5; Figure 17

$^{13}$C-NMR: $\delta$ ppm, 100 MHz, acetone-$d_6$; see Table 5; Figure 18

3.5.3 Compound 3 [(−)−syringaresinol]

**Compound 3** was obtained as a white amorphous solid, soluble in acetone (10 mg, 0.00033% based on dried weight of stems).

HR-ESI-MS: [M+Na]$^+$ at m/z 441.1540 (calculated for C$_{22}$H$_{26}$NO$_8$Na, 441.1520); Figure 23

$[^]{\text{[\alpha]}^\circ}_{\text{D}}$: $-31.0^\circ$ (c 0.01, MeOH)

FT-IR: $\nu_{\text{max}}$ (ATR) cm$^{-1}$: 3397, 2939, 2835, 1610, 1515, 1459, 1424, 1332, 1212, 1108; Figure 24
3.5.4 Compound 4 [(+)-ampelopsin A)]

Compound 4 was obtained as a red amorphous solid, soluble in MeOH and acetone (2.4 mg, 0.00008% based on dried weight of stems).

HR-ESI-MS: \([M+H]^+\) at \(m/z\) 471.1455 (calculated for \(C_{28}H_{22}NO_7\), 471.1444); Figure 33

\([\alpha]^{25}_D\): +98° (c 0.10, MeOH)

FT-IR: \(v_{\text{max}}\) (ATR) \(\text{cm}^{-1}\): 3314, 1597, 1513, 1451, 1339, 1234, 1173, 1151, 1135, 1007, 834; Figure 34

\(^1\)H-NMR: \(\delta\) ppm, 400 MHz, acetone-\(d_6\); see Table 6; Figure 25-26

\(^{13}\)C-NMR: \(\delta\) ppm, 100 MHz, acetone-\(d_6\); see Table 6; Figure 27

3.5.5 Compound 5 [(-)-pauciflorol E)]

Compound 5 was obtained as a green amorphous solid, soluble in acetone (3.0 mg, 0.0001% based on dried weight of stems).

HR-ESI-MS: \([M+H]^+\) ion at \(m/z\) 469.1320 (calculated for \(C_{28}H_{20}NO_7\), 469.1287); Figure 45

\([\alpha]^{25}_D\): -166° (c 0.10, MeOH)

FT-IR: \(v_{\text{max}}\) (ATR) \(\text{cm}^{-1}\): 3339, 2924, 2852, 1656, 1596, 1531, 1447, 1374, 1333, 1261, 1175, 1158, 1110, 1010, 835; Figure 46

\(^1\)H-NMR: \(\delta\) ppm, 400 MHz, acetone-\(d_6\); see Table 7; Figure 35-36

\(^{13}\)C-NMR: \(\delta\) ppm, 100 MHz, acetone-\(d_6\); see Table 7; Figure 37-38

3.5.6 Compound 6 [(+)-maeruanitrile A)]

Compound 6 was obtained as a reddish-brown amorphous solid, soluble in acetone (2.4 mg, 0.00003% based on dried weight of roots).
3.5.7 Compound 7 (maeruanitrile B)

Compound 7 was obtained as a reddish-brown amorphous solid, soluble in acetone (1.4 mg, 0.00002% based on dried weight of roots).

HR-ESI-MS : [M+Na]$^+$ at m/z 241.0585 (calculated for C$_{11}$H$_{10}$N$_2$O$_3$Na, 241.0584); Figure 58

$[^{25}\alpha]_{D}^2$ : +3.0° (c 0.001, MeOH)

UV : $\lambda_{\text{max}}$ (MeOH) nm (log $\varepsilon$): 218 (5.49), 268 (4.66), 322 (4.04); Figure 57

CD : (c 0.000045, MeOH) nm (mdeg): 240 (+8.20), 265.5 (−9.64), 283.0 (0.07); Figure 60

FT-IR : $\nu_{\text{max}}$ (ATR) cm$^{-1}$: 3291, 2256, 1789, 1629, 1462, 1342, 1722; Figure 59

$^1$H-NMR : $\delta$ ppm, 600 MHz, acetone-d$_6$; see Table 9; Figure 61

$^{13}$C-NMR : $\delta$ ppm, 150 MHz, acetone-d$_6$; see Table 9; Figure 62

3.5.8 Compound 8 (maeroxime A)

Compound 8 was obtained as a reddish-brown amorphous solid, soluble in acetone (2.4 mg, 0.00003% based on dried weight of roots).

HR-ESI-MS : [M+Na]$^+$ at m/z 271.051 (calculated for C$_{12}$H$_{12}$N$_2$O$_2$SNa, 271.0517); Figure 69

UV : $\lambda_{\text{max}}$ (MeOH) nm (log $\varepsilon$): 228 (4.85), 300 (4.39), 342 (3.77); Figure 68

FT-IR : $\nu_{\text{max}}$ (ATR) cm$^{-1}$: 3163, 2924, 2850, 2360, 2249, 1626, 1451, 1298, 1208, 1160, 1022; Figure 70

$^1$H-NMR : $\delta$ ppm, 600 MHz, CD$_3$OD; see Table 10; Figure 71

$^{13}$C-NMR : $\delta$ ppm, 150 MHz, CD$_3$OD; see Table 10; Figure 72
UV : $\lambda_{\text{max}}$ (MeOH) nm $\log \varepsilon$: 212 (5.12), 217 (5.26), 225 (4.80), 269 (4.15); Figure 80

FT-IR : $\nu_{\text{max}}$ (ATR) cm$^{-1}$: 3369, 2923, 2852, 1714, 1627, 1501, 1457, 1337, 1198, 1093; Figure 82

$^1$H-NMR : $\delta$ ppm, 400 MHz, DMSO-$d_6$; see Table 11; Figure 83-84

$^{13}$C-NMR : $\delta$ ppm, 100 MHz, DMSO-$d_6$; see Table 11; Figure 85

3.5.9 Compound 9 (maeroxime B)

Compound 9 was obtained as an orange-brown amorphous solid, soluble in acetone (1.0 mg, 0.00001% based on dried weight of roots).

HR-ESI-MS : [M+H]$^+$ at $m/z$ 279.0780 (calculated for C$_{13}$H$_{14}$N$_2$O$_3$S, 279.0803); Figure 96

UV : $\lambda_{\text{max}}$ (MeOH) nm $\log \varepsilon$: 212 (4.69), 280 (3.25), 314 (4.18); Figure 94

FT-IR : $\nu_{\text{max}}$ (ATR) cm$^{-1}$: 3283, 2924, 2851, 1718, 1617, 1521, 1421, 1241, 1197, 1074, 1032; Figure 95

$^1$H-NMR : $\delta$ ppm, 400 MHz, DMSO-$d_6$; see Table 12; Figure 97

$^{13}$C-NMR : $\delta$ ppm, 100 MHz, DMSO-$d_6$; see Table 12; Figure 98

3.5.10 Compound 10 (maeroxime C)

Compound 10 was obtained as a yellow amorphous solid, soluble in acetone (3.5 mg, 0.00005% based on dried weight of roots).

HR-ESI-MS : [M+H]$^+$ at $m/z$ 279.0782 (calculated for C$_{13}$H$_{14}$N$_2$O$_3$S, 279.0803); Figure 106

UV : $\lambda_{\text{max}}$ (MeOH) nm $\log \varepsilon$: 208 (4.80), 272 (4.47), 316 (4.15); Figure 105

FT-IR : $\nu_{\text{max}}$ (ATR) cm$^{-1}$: 3306, 2956, 2924, 2854, 1729, 1618, 1461, 1378, 1283, 1074, 1037; Figure 107

$^1$H-NMR : $\delta$ ppm, 400 MHz, DMSO-$d_6$; see Table 13; Figure 108


\(^{13}\text{C}-\text{NMR}\) : \(\delta \text{ ppm, 100 MHz, DMSO-}d_6\); see Table 13; Figure 109

3.5.11 Compound 11 (maeruabisindole A)

Compound 11 was obtained as a pale green amorphous solid, soluble in MeOH (1.1 mg, 0.00002% based on dried weight of roots).

HR-ESI-MS : \([M+H]^+ \text{ at } m/z 390.1298 \) (calculated for \(C_{22}H_{20}N_3O_3S, 390.1271\)); Figure 116

UV : \(\lambda_{\text{max}} \text{ (MeOH) nm (log } \varepsilon) : 210 (3.72), 270 (4.03), 315 (3.95), 355 (3.49),
365 (3.44); Figure 115

FT-IR : \(\nu_{\text{max}} \text{ (ATR) cm}^{-1} : 3384, 2919, 2850, 1625, 1559, 1508, 1458, 1420, 1325,
1286, 1246, 1228, 1196, 1162, 1089, 1029; Figure 117

\(^1\text{H}-\text{NMR}\) : \(\delta \text{ ppm, 400 MHz, DMSO-}d_6\); see Table 14; Figure 118-119

\(^{13}\text{C}-\text{NMR}\) : \(\delta \text{ ppm, 100 MHz, DMSO-}d_6\); see Table 14; Figure 120-121

3.5.12 Compound 12 (maeruabisindole B)

Compound 12 was obtained as a pale green amorphous solid, soluble in MeOH (1.1 mg, 0.00002% based on dried weight of roots).

HR-ESI-MS : \([M+H]^+ \text{ at } m/z 406.1224 \) (calculated for \(C_{22}H_{20}N_3O_3S, 406.1220\)); Figure 130

UV : \(\lambda_{\text{max}} \text{ (MeOH) nm (log } \varepsilon) : 210 (4.47), 230 (4.15), 310 (3.93), 340 (3.31),
355 (3.31); Figure 129

FT-IR : \(\nu_{\text{max}} \text{ (ATR) cm}^{-1} : 3396, 2921, 2851, 1602, 1465, 1377, 1258, 1172, 1117,
1025; Figure 131

\(^1\text{H}-\text{NMR}\) : \(\delta \text{ ppm, 400 MHz, CD}_3\text{OD}; \text{ see Table 15; Figure 132-133}

\(^{13}\text{C}-\text{NMR}\) : \(\delta \text{ ppm, 100 MHz, CD}_3\text{OD}; \text{ see Table 15; Figure 134}
3.5.13 Compound 13 (maeruabisindole C)

Compound 13 was obtained as a dark green amorphous solid, soluble in acetone (3.5 mg, 0.00005% based on dried weight of roots).

HR-ESI-MS : [M-H]⁻ at m/z 326.0968 (calculated for C₂₀H₁₂N₃O₂, 326.0935); Figure 141

UV : \( \lambda_{\text{max}} \) (MeOH) nm (log \( \epsilon \)): 210 (4.07), 285 (2.93), 355 (2.21), 365 (2.36); Figure 142

FT-IR : \( \nu_{\text{max}} \) (ATR) cm⁻¹: 3359, 3192, 2921, 2851, 2212, 1658, 1632, 1468, 1412, 1279, 1135, 702, 632; Figure 143

\(^{1}H\)-NMR : \( \delta \) ppm, 400 MHz, acetone-\( d_6 \); see Table 16; Figure 144-145

\(^{13}C\)-NMR : \( \delta \) ppm, 100 MHz, acetone-\( d_6 \); see Table 16; Figure 146-147

3.6 Evaluation of inhibitory activity on nitric oxide (NO) production and cytotoxicity

RAW 264.7 macrophage cells (ATCC, TIB-71) were cultivated in DMEM (Dulbecco’s Modified Eagle Medium, Gibco, Thermo Fisher Scientific, Waltham, MA, USA) including 10% fetal bovine serum (FBS), and penicillin (100 U/mL) and streptomycin (100 µg/mL) in a humidified atmosphere (37°C, 5%CO₂). Indomethacin was used as positive control.

Briefly, cells were placed in 96-well plate (5 \times 10^⁴ cells/well) and pre-treated with various concentrations of compounds for 24 h. Then, the cells were added with 100 ng/mL LPS and further incubated for 24 h. After that, the supernatants were collected for NO production assay and the cytotoxicity was determined by MTT assay.

Cells were pre-treated with various concentrations of samples for 24 h. Cells were induced with 100 ng/mL LPS for 24 h. The culture supernatant was collected for NO production analysis using Griess reagent kit (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA), and cells were further examined for their viability.

Ninety µL of supernatant was mixed with 10 µL of Griess reagent and incubated for 30 min at room temperature, then the NO concentration was measured at 540 nm
using the microplate reader (Synergy H1, BioTek, Santa Clara, CA, USA) and calculated using NaNO₂ standard curve (Kim et al., 2020). Percentage of NO production was calculated from equation 1.

\[
\text{% NO production} = \frac{A}{B} \times 100 \quad \text{equation 1}
\]

\( A \) = concentration of nitric oxide in cells induced by LPS with sample pre-treatment

\( B \) = concentration of nitric oxide in cells induced by LPS without sample pre-treatment

The NO inhibitory activity was indicated as half maximal inhibitory concentration (IC₅₀) calculated by GraphPad Prism 9.

For MTT assay, MTT solution (1 mg/mL) was added to each well and incubated for 4 h at 37°C. After removal of the MTT solution, cells were added with DMSO to dissolve formazan product. The absorbance was measured at 570 nm using a microplate reader (Eaknai et al., 2022).

3.7 Statistical analysis

The results were expressed as mean ± standard error of the mean (SEM) from three independent experiments. The mean differences of IC₅₀ values of samples vs that of indomethacin (positive control) were analyzed by one-way analysis of variance (ANOVA) (GraphPad Prism 9). Statistical significance was defined as \( p < 0.05 \).
CHAPTER IV
RESULTS AND DISCUSSION

In this study, eight compounds were isolated from the EtOAc extract of the stems of *Capparis micracantha* and five compounds were obtained from the n-butanol extract of the roots of *Maerua siamensis*. They were purified by chromatographic techniques and their chemical structures were identified or elucidated by spectroscopic techniques including IR, UV, MS and NMR spectroscopy. The extracts and all isolated compounds were tested for their inhibitory effect on nitric oxide production.

4.1 Identification of compounds isolated from *Capparis micracantha* stems

4.1.1 Identification of compound 1 (methyl 6-methoxy-3-indolecarbonate)

Compound 1 was obtained as a yellow amorphous solid. Its pseudo-molecular \([M+H]^+\) ion was observed in the high resolution ESI mass spectrum at \(m/z\) 206.0818 (calculated for \(C_{11}H_{12}NO_3\), 206.0817) (Figure 5), suggesting a molecular formula of \(C_{11}H_{11}NO_3\), with seven degrees of unsaturation. The IR spectrum showed NH absorption peak at 3307 cm\(^{-1}\) and conjugated ester carbonyl peak at 1679 cm\(^{-1}\) (Figure 6).

The \(^1\)H NMR spectrum of compound 1 (400 MHz, acetone-\(d_6\)) (Figure 7-8 and Table 4) displayed the signals of one carboxymethyl group at \(\delta_H 3.82\) (3H, s, 8-COOCH\(_3\)) and one methoxy group at \(\delta_H 3.81\) (3H, s, 6-OCH\(_3\)), an ABX system of a 1,2,4 trisubstituted aromatic ring at \(\delta_H 6.86\) (1H, dd, \(J = 8.7\), 2.4 Hz, H-5), 7.03 (1H, \(d, J = 2.4\) Hz, H-7) and 7.92 (1H, \(d, J = 8.7\) Hz, H-4), an olefinic methine proton at \(\delta_H 7.90\) (1H, s, H-2) and a broad NH proton at \(\delta_H 10.76\) (1H, br s, NH-1). These data were indicative of an indole nucleus with two substituents.

Its \(^{13}\)C NMR data (100 MHz, acetone-\(d_6\)) (Figure 9 and Table 4) and \(^1\)H-\(^{13}\)C HSQC spectrum (Figure 10) showed resonances of eleven carbon atoms including a methoxy carbon 55.8 (6-OCH\(_3\)), a carboxymethyl group at \(\delta_C 50.9\) (8-COOCH\(_3\)) and 168.4 (C-8), three aromatic methine carbons at \(\delta_C 95.9\) (C-7), 112.4 (C-5) and 122.5 (C-4), an olefinic
methane carbon at $\delta_c$ 131.5 (C-2), and four quaternary carbons at $\delta_c$ 108.6 (C-3), 121.2 (C-3a), 138.5 (C-7a) and 157.9 (C-6).

The $^1$H-$^1$H COSY spectrum (Figure 13) exhibited cross peak between the signals of H-4 at $\delta_h$ 7.92 and H-5 at $\delta_h$ 6.86, confirming their ortho-coupling. The assignment of H-4 signal was confirmed by its long-range $^1$H-$^{13}$C HMBC correlation (Figure 11-12 and Table 4) to that of C-3. The methoxy substitution at C-6 was confirmed by $^1$H-$^1$H NOESY cross peaks (Figure 14) between both H-5 and H-7 signals with that of 6-OCH$_3$, as well as HMBC cross peak of this methoxy protons with the signal of C-6. Additionally, the olefinic H-2 signal at $\delta_h$ 7.90 showed HMBC cross peaks with the signals of C-3a and C-7a, establishing a substituent at position 3 of the indole nucleus. Then spectroscopic data indicated an indole moiety with a methoxy substitution at C-6 and an ester carbonyl substitution at C-3. These NMR data helped identify compound 1 as methyl 6-methoxy-3-indolecarbonate, which has previously been found in the roots and rhizomes of Clematis manshurica (family Ranunculaceae), which are used as an anti-inflammatory, analgesic and antitumor herb in traditional Chinese medicine (Shi et al., 2006).
Table 4. $^1$H- and $^{13}$C-NMR data of compound 1 (400 MHz, in acetone-$d_6$) and methyl 6-methoxy-3-indolecarbonate (300 MHz, in CDCl$_3$)

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<td>50.9</td>
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*Shi et al. (2006).

Figure 5. HR-ESI mass spectrum of compound 1
Figure 6. IR spectrum of compound 1

Figure 7. $^1$H-NMR spectrum of compound 1 (400 MHz, acetone-$d_6$)
Figure 8. $^1$H-NMR spectrum of compound 1 (expansion between $\delta_H$ 6.5-8.5 ppm)

Figure 9. $^{13}$C-NMR spectrum of compound 1 (100 MHz, acetone-$d_6$)
Figure 10. $^1$H-$^{13}$C HSQC spectrum of compound 1

Figure 11. $^1$H-$^{13}$C HMBC spectrum of compound 1
Figure 12. $^1\text{H}-^{13}\text{C}$ HMBC spectrum of compound 1
(expansion between $\delta_\text{H}$ 6.3-8.6 ppm, $\delta_\text{C}$ 70-160 ppm)

Figure 13. $^1\text{H}-^1\text{H}$ COSY spectrum of compound 1
Figure 14. $^1$H-$^1$H NOESY spectrum of compound 1

4.1.2 Identification of compound 2 (vanillic acid)

Compound 2 was obtained as a white amorphous solid soluble in acetone. Its HR-ESI mass spectrum (Figure 15) revealed a pseudo-molecular [M+H]$^+$ ion peak at m/z 169.0504, in accordance with the molecular formula C$_8$H$_8$O$_4$ (calculated for C$_8$H$_9$O$_4$, 169.0501). The IR spectrum (Figure 16) displayed absorption peaks of hydroxyl group at 3483 cm$^{-1}$ and conjugated carboxyl group at 1674 cm$^{-1}$.

In the $^1$H NMR spectrum (400 MHz, acetone-$d_6$) (Figure 17 and Table 5), an ABX system of three aromatic protons were observed at $\delta_H$ 6.91 (1H, d, J = 8.2 Hz, H-5), 7.56 (1H, d, J = 2.0 Hz, H-2) and 7.59 (1H, dd, J = 8.2, 2.0 Hz, H-6). A three-proton resonance of one methoxy group was also located at $\delta_H$ 3.90 (3H, s, 3-OCH$_3$).

Its $^{13}$C NMR spectrum (100 MHz, acetone-$d_6$) (Figure 18 and Table 5) exhibited eight carbon signals including those of three methines at $\delta_C$ 113.5 (C-2), 115.6 (C-5) and 124.9 (C-6), three quaternary carbons at $\delta_C$ 123.0 (C-1), 148.1 (C-3) and 152.1 (C-4), a carboxylic acid carbonyl at $\delta_C$ 167.6 (C-7) and a methoxy carbon at $\delta_C$ 56.4 (3-OCH$_3$).
These spectroscopic data of compound 2 was indicative of its chemical structure as a 1,3,4-trisubstituted benzene ring. One substituent is a carboxylic acid group which could be located at position 1, based on the long-range $^1$H-$^{13}$C HMBC cross peaks from the proton signals of both H-2 and H-6 to that of C-7 (Figure 20). A methoxy substituent at position 3 was confirmed by $^1$H-$^1$H NOESY correlation (Figure 22) observed between the proton signals of H-2 and 3-OCH$_3$. Therefore, the third substituent group, which is a hydroxy group, could be placed at position 4 of the benzene ring. These data helped identify compound 2 as vanillic acid. This aromatic compound has been isolated from several plants; for example, from the roots of *Lepidium meyenii* (family Brassicaceae) (Bai et al., 2015), the aerial parts of *Alyssum alyssoides* (family Brassicaceae) (Tsiftsoglou et al., 2019) and the aerial parts of *Matthiola incana* (family Brassicaceae). Recently, vanillic acid has been reported to exhibit anti-inflammatory activity in osteoarthritis through inhibition of inflammatory cytokines such as interleukin 1β (IL-1β) and tumor necrosis factor α (TNF-α) in human chondrocytes (Ziadlou et al., 2020).

![ vanillic acid ]

O
C
H
3
O
C
H
O
3
2
1
7
6
5

vanillic acid
Table 5. $^1$H- and $^{13}$C-NMR data of compound 2 (400 MHz, in acetone-$d_6$) and vanillic acid (500 MHz, in CD$_3$OD)

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<th>vanillic acid*</th>
<th>HMBC correlation with</th>
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<td>$\delta_H$ (mult., $J$ in Hz)</td>
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<td>7.57 ($d$, 2.0)</td>
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<td>-</td>
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<td>-</td>
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<td>5</td>
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<td>115.6</td>
<td>7.12 ($d$, 8.2)</td>
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<tr>
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<td>3.90 (s)</td>
<td>56.4</td>
<td>3.91 (s)</td>
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</table>

*Chang et al. (2009).

Figure 15. HR-ESI mass spectrum of compound 2
Figure 16. IR spectrum of compound 2

Figure 17. $^1$H-NMR spectrum of compound 2 (400 MHz, acetone-$d_6$)
Figure 18. $^{13}$C-NMR spectrum of compound 2 (100 MHz, acetone $d_6$)

Figure 19. $^1$H-$^{13}$C HSQC spectrum of compound 2
Figure 20. $^1$H-$^{13}$C HMBC spectrum of compound 2

Figure 21. $^1$H-$^1$H COSY spectrum of compound 2
4.1.3 Identification of compound 3 [(-)-syringaresinol]

Compound 3 was obtained as a white amorphous solid, which gave a quenching spot under short-wave UV light (254 nm) and appeared as a black spot after spraying with Ce₂(SO₄)₃ and heated. Its molecular formula was deduced as C₂₂H₂₆O₈, based on a pseudo-molecular [M+Na]⁺ ion peak observed at m/z 441.1540 in the HR-ESI mass spectrum (calculated for C₂₂H₂₆NO₈Na, 441.1520) (Figure 23). The IR spectrum of compound 3 (Figure 24) showed hydroxyl absorption peak at 3397 cm⁻¹. In the ¹³C-NMR (100 MHz, CDCl₃) (Figure 27) and ¹H-¹³C HSQC spectra (Figure 28), only eight carbon signals were observed, suggesting its symmetrical structure. The signals were those of four methoxy carbons which resonated at $\delta_C$ 56.4 (3’/3’’-OCH₃ and 5’/5’’-OCH₃), two methine carbons at $\delta_C$ 54.3 (C-1 and C-5), two oxymethines at $\delta_C$ 86.1 (C-2 and C-6), two oxymethylene at $\delta_C$ 71.8 (C-4 and C-8), four aromatic methine carbons at $\delta_C$ 102.8 (C-2’/2’’ and C-6’/6’’), and eight aromatic quaternary carbons at $\delta_C$ 132.1 (C-1’/1’’), 134.3 (C-4’/4’’), and 147.1 (C-3’/3’’ and C-5’/5’’).
Its $^1$H NMR spectrum (400 MHz, CDCl$_3$) (Figure 25-26 and Table 6) displayed resonances of methine protons at $\delta_H$ 3.09 (2H, $m$, H-1, H-5), oxymethylene protons at $\delta_H$ 3.91 (2H, s, H$_b$-4/8), $\delta_H$ 4.23 (2H, $ddd$, $J= 9.2, 6.8, 2.2$ Hz, H$_a$-4/8), oxymethine protons at $\delta_H$ 4.73 (2H, $d$, $J= 2.8$ Hz, H-2$'$/2$''$ and H-6$'$/6$''$), methoxy groups at $\delta_H$ 3.91 (6H, $s$, 3$'$/3$''$-OCH$_3$) and 3.91 (6H, $s$, 5$'$/5$''$-OCH$_3$) and hydroxy protons at $\delta_H$ 5.51 (2H, $s$, 4$'$/4$''$-OH).

In $^1$H-$^{13}$C HMBC spectrum (Figure 29-30), correlations between $\delta_H$ 4.74 (H-2 and H-6) with C-4/8 ($\delta_C$ 71.8), C-2$'$/2$''$ and C-6$'$/6$''$ ($\delta_C$ 102.8) indicated the connection of two phenylpropanoid subunits. In addition, $^1$H-$^1$H NOESY cross peaks (Figure 32) of H-2$'$/2$''$/6$'$/6$''$ with 3$'$/3$''$/5$'$/5$''$-OCH$_3$ helped establish the substitution of methoxy groups at positions 3$'$, 3$''$, 5$'$ and 5$''$, hence hydroxy groups at positions 4$'$ and 4$''$. Moreover, the $^1$H-$^1$H COSY cross peaks (Figure 31) indicated the connection of H-2/6, H-1/5 and H-4/8.

These spectroscopic data suggested the chemical structure of compound 3 as a tetrahydrofuran lignan, compared to the previously reported (Chen et al., 1998).

Therefore, compound 3 was identified as (−)-syringaresinol, which was firstly isolated from the stems of Annona cherimola (family Annonaceae). Later, it has been found in the orchid plants such as Dendrobium secundum and Dendrobium heterocarpum (Sritularak et al., 2011; Warinhomhoun et al., 2021). (−)-syringaresinol showed inhibitory activity on nitric oxide production and LPS-induced NF-κB activation in a BV2 microglia cells (Zhang et al., 2022).
Table 6. $^1$H- and $^{13}$C-NMR data of compound 3 (400 MHz, in CDCl$_3$) and (−)-syringaresinol (400 MHz, in CDCl$_3$).

<table>
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<th>Compound 3</th>
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<th>HMBC correlation with</th>
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<td>$\delta_C$</td>
<td>$\delta_H$ (mult., $J$ in Hz)</td>
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<td>3.11 (m)</td>
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<td>86.1</td>
<td>4.74 (d, 4.3)</td>
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<td>4/8</td>
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<td>71.8</td>
<td>4.29 (d, 9.6, 8.8)</td>
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<tr>
<td></td>
<td>3.91 (s)</td>
<td></td>
<td>3.91 (d, 9.6, 3.6)</td>
</tr>
<tr>
<td>1'/1''</td>
<td>-</td>
<td>132.1</td>
<td>-</td>
</tr>
<tr>
<td>2'/2''</td>
<td>6.59 (d, 2.8)</td>
<td>102.8</td>
<td>6.59 (s)</td>
</tr>
<tr>
<td>3'/3''</td>
<td>-</td>
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<tr>
<td>4'/4''</td>
<td>-</td>
<td>134.3</td>
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<td>6'/6''</td>
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<td>6.59 (s)</td>
</tr>
<tr>
<td>4'/4''-OH</td>
<td>5.51 (s)</td>
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<td>3'/3''-OCH$_3$</td>
<td>3.91 (s)</td>
<td>56.4</td>
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<td>5'/5''-OCH$_3$</td>
<td>3.91 (s)</td>
<td>56.4</td>
<td>3.91 (s)</td>
</tr>
</tbody>
</table>

Figure 23. HR-ESI mass spectrum of compound 3

Figure 24. IR spectrum of compound 3
Figure 25. $^1$H-NMR spectrum of compound 3 (400 MHz, CDCl$_3$)

Figure 26. $^1$H-NMR spectrum of compound 3 (expansion between $\delta_H$ 4.2-6.9 ppm)
Figure 27. $^{13}$C-NMR spectrum of compound 3 (100 MHz, CDCl$_3$)

Figure 28. $^1$H-$^{13}$C HSQC spectrum of compound 3
Figure 29. $^1H$-$^13C$ HMBC spectrum of compound 3

Figure 30. $^1H$-$^13C$ HMBC spectrum of compound 3
(expansion between $\delta_H$ 1.0-3.3 ppm, $\delta_C$ 55-145 ppm)
Figure 31. $^1$H-$^1$H COSY spectrum of compound 3

Figure 32. $^1$H-$^1$H NOESY spectrum of compound 3
4.1.4 Identification of compound 4 [(+)-ampelopsin A]

Compound 4 was obtained as a red amorphous solid. Its molecular formula was determined as C$_{28}$H$_{22}$O$_{7}$ (eighteen degrees of unsaturation) based on an observed pseudo-molecular [M+H]$^+$ ion peak at m/z 471.1455 (calculated for C$_{28}$H$_{22}$O$_{7}$, 471.1444) in the HR-ESI mass spectrum (Figure 33). The IR spectrum of this compound showed hydroxyl absorption peak at 3397 cm$^{-1}$ (Figure 34).

The $^1$H NMR spectrum (400 MHz, CD$_3$OD) of compound 4 (Figure 35-36 and Table 7) displayed the resonances of two pairs of ortho-coupled protons of two para-disubstituted benzene rings at $\delta_H$ 6.83 (2H, d, $J = 8.4$ Hz, H-2a/6a) and 6.59 (2H, $J = 8.4$ Hz, H-3a/5a), and $\delta_H$ 6.70 (2H, d, $J = 8.4$ Hz, H-3b/5b) and 7.02 (2H, $J = 8.4$ Hz, H-2b/6b), two pairs of meta-coupled protons at $\delta_H$ 6.12 (1H, d, $J = 2.4$ Hz, H-12a) and 6.53 (1H, d, $J = 2.4$ Hz, H-14a), and $\delta_H$ 6.11 (1H, d, $J = 2.4$ Hz, H-14b) and 6.32 ppm (1H, d, $J = 2.4$ Hz, H-12b). These data implied the presence of four aromatic rings. Two pairs of vicinal aliphatic methine protons were also observed at $\delta_H$ 5.38 ppm (2H, s, H-7a/8a) and $\delta_H$ 4.03 (1H, d, $J = 11.6$ Hz, H-8b) and 5.70 (1H, d, $J = 11.6$ Hz, H-7b). The number of carbon atoms in the molecular formula and the number of aromatic rings were deduced from $^1$H NMR data corresponding to the basic structure of a stilbenoid dimer.

Twenty-four carbon resonances (100 MHz, CD$_3$OD) were appeared in $^{13}$C NMR spectrum (Figure 37-38 and Table 7). These carbon signals could be differentiated, with the aid of $^1$H-$^{13}$C HSQC spectrum (Figure 39), into those of twelve quaternary carbons including six oxygen-substituted ones at $\delta_C$ 71.9 (C-8a), 119.2 (C-10b), 129.0 (C-10a), 133.3 (C-1b), 139.9 (C-9a), 143.6 (C-9b), 156.4 (C-4a), 157.7 (C-11b), 159.3 (C-4b), 159.5 (C-13a/13b) and 160.6 (C-11a), eight aromatic methine carbons at $\delta_C$ 97.7 (C-12a), 101.8 (C-12b), 105.6 (C-14b), 111.0 (C-14a), 115.8 (C-3a/5a and C-3b/5b) and 129.2 (C-2a/6a and C-2b/6b) and four aliphatic methine carbons at $\delta_C$ 44.2 (C-7a), 50.0 (C-8b), 71.9 (C-8a) and 89.4 (C-7b).

Two aromatic rings of one stilbenoid subunit was connected via the methine carbons 7a and 8a, as confirmed by long-range $^1$H-$^{13}$C HMBC cross peaks (Figure 40-42 and Table 7) of H-7a ($\delta_H$ 5.38) with C-2a/6a ($\delta_C$ 129.2) and C-9a ($\delta_C$ 139.9) and of H-
8a (δ_H 5.38) with C-10a (δ_C 120.0) and C-14a (δ_C 111.0). HMBC correlations were also observed between H-7a signal with carbon signals of another stilbenoid subunit at C-9b (δ_C 143.6), C-10b (δ_C 119.2) and C-11b (δ_C 157.7). The second stilbenoid subunit displayed HMBC cross peaks between H-7b (δ_H 5.70) and C-2b/6b (δ_C 129.2) and C-9b (δ_C 143.6), as well as between H-8b (δ_H 4.03 ppm) and C-9a and C-10a. These data suggested that the compound comprised two resveratrol subunits which were connected via a seven-membered ring and a furan ring, with a hydroxyl substituent at position 8a. The configuration of H-7a, H-8a, H-7b and H-8b was assigned based on comparison of the optical rotation of this compound (+228.0° (c 0.1, MeOH)) with previous study (+98.0° (c 0.1, MeOH)) (Oshima et al., 1990). Therefore, compound 4 was identified as (+)-ampelopsin A, which has been previously isolated from the roots of Ampelopsis brevipedunculata var. hancei (Oshima et al., 1990) and Vitis vinifera (family Vitaceae). It was reported to possess neuroprotective ability by increasing the function of the central or peripheral nervous system (Hong et al., 2021). The compound also demonstrated anti-inflammatory effect against lipopolysaccharide (LPS)-induced arthritis (Wang et al., 2011).
(+)-Ampelopsin A
Table 7. $^1$H- and $^{13}$C-NMR data of compound 4 (400 MHz, in CD$_3$OD) and (+)-ampelopsin A (500 MHz, in acetone-$d_6$)

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<td>$\delta_C$ (mult., J in Hz)</td>
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<td>129.2</td>
<td>6.90 (d, 8.3)</td>
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Figure 33. HR-ESI mass spectrum of compound 4

Figure 34. IR spectrum of compound 4
Figure 35. $^1$H-NMR spectrum of compound 4 (400 MHz, CD$_3$OD)

Figure 36. $^1$H-NMR spectrum of compound 4 (expansion between $\delta_H$ 5.2-7.2 ppm)
Figure 37. $^{13}$C-NMR spectrum of compound 4 (100 MHz, CD$_3$OD)

Figure 38. $^{13}$C-NMR spectrum of compound 4 (expansion between $\delta_c$ 0-165 ppm)
Figure 39. $^1$H-$^{13}$C HSQC spectrum of compound 4

Figure 40. $^1$H-$^{13}$C HMBC spectrum of compound 4
Figure 41. $^1$H-$_{13}$C HMBC spectrum of compound 4
(expansion between $\delta_H$ 4.0-7.1 ppm, $\delta_C$ 128-162 ppm)

Figure 42. $^1$H-$_{13}$C HMBC spectrum of compound 4
(expansion between $\delta_H$ 3.9-7.2 ppm, $\delta_C$ 103-163 ppm)
Figure 43. $^1$H-$^1$H COSY spectrum of compound 4

Figure 44. $^1$H-$^1$H NOESY spectrum of compound 4
4.1.5 Identification of compound 5 [(-)-pauciflorol E]

Compound 5 was obtained as a yellow amorphous solid. Its molecular formula was established as C_{28}H_{20}O_{7} (nineteen degrees of unsaturation) based on the pseudo-molecular [M+H]^+ ion peak in the high-resolution ESI mass spectrum (Figure 45) at m/z 469.1320 (calculated for C_{28}H_{21}O_{7}, 469.1287). The IR spectrum (Figure 46) showed absorption bands of hydroxyl (3339 cm\(^{-1}\)) and conjugated keto carbonyl (1656 cm\(^{-1}\)) functionalities. These data suggested the similarity between this compound and compound 4, except the presence of a keto carbonyl group instead of a hydroxyl group.

The \(^1\)H NMR spectrum (400 MHz, acetone-d\(_6\)) (Figure 47-49 and Table 8) exhibited signals of two para-disubstituted benzene rings at \(\delta_H\) 6.77 (2H, dd, J = 8.6, 1.2 Hz, H-2a/6a) and 6.70 (2H, \(J = 8.6 \text{ Hz, H-3a/5a}\)), and at \(\delta_H\) 6.83 (2H, \(d, J = 8.6 \text{ Hz, H-3b/5b}\)) and 7.18 (2H, \(d, J = 8.6 \text{ Hz, H-2b/6b}\)), two pairs of meta-coupled protons at \(\delta_H\) 6.45 (1H, \(d, J = 2.4 \text{ Hz, H-12a}\)) and 7.12 (1H, \(d, J = 2.4 \text{ Hz, H-14a}\)), and at \(\delta_H\) 6.39 (1H, \(br \ s, H-14b\)) and 6.49 (1H, \(d, J = 2.0 \text{ Hz, H-12b}\)). These data were similar to those of compound 4. The rest were signals of an aliphatic methine proton at \(\delta_H\) 6.05 (1H, \(br \ s, H-7a\)) and a set of mutually coupled aliphatic methine protons at \(\delta_H\) 5.94 (1H, \(d, J = 10.8 \text{ Hz, H-7b}\)) and 4.52 (1H, \(d, J = 10.8 \text{ Hz, H-8b}\)).

Twenty-four carbon resonances representing 28 carbon atoms were observed in the \(^{13}\)C NMR spectrum (100 MHz, acetone-d\(_6\)) (Figure 50-51 and Table 8). Most of these signals were similar to those of compound 4 except for the presence of a keto carbonyl resonance at \(\delta_C\) 195.6 (C-8a) instead of a methine carbon as compound 4. The position of this C-8a carbonyl carbon could be confirmed with \(^1\)H-\(^{13}\)C HMBC cross peaks (Figure 53-54) of the methine H-7a signal (\(\delta_H\) 6.05) and the aromatic H-14a proton (\(\delta_H\) 7.12) with the signal of keto carbonyl at \(\delta_C\) 195.6. In addition, HMBC spectrum also showed correlations of H-7a with C-10b (\(\delta_C\) 114.3), C-2a/6a (\(\delta_C\) 128.4), C-9a (\(\delta_C\) 134.1) and C-9b (\(\delta_C\) 142.5), confirming their connectivity as same as the compound 4. Furthermore, significant long-range HMBC cross peaks were observed between H-8b signal (\(\delta_H\) 4.53) with C-14b (\(\delta_C\) 105.8), C-10b, C-10a (\(\delta_C\) 124.4), C-1b (\(\delta_C\)
130.5) and C-9a ($\delta_\text{C} 134.1$), as well as from H-7b signal (at $\delta_\text{H} 5.94$) to C-2b/6b ($\delta_\text{C} 129.9$) and C-9b. $^1\text{H}-^1\text{H}$ NOESY experiment (Figure 56) confirmed a relative configuration between H-7b and H-8b as $\text{trans}$, from the observed cross peaks between H-7b and H-14b signals, and between H-8b and H-2b/6b signals. Therefore, compound 5 was identified as a resveratrol dimer pauciflorol E, previously found as a constituent of the stem bark of $Vatica$ $pauciflora$ (family Dipterocarpaceae). The optical rotation of this compound was $-166.0^\circ$ (c 0.1, MeOH)) compared with previous study ($-228.0^\circ$ (c 0.1, MeOH)) (Ito et al., 2004). Resveratrol and its oligomers have been reported to display several bioactivities e.g., cytotoxic, antibacterial, and anti-HIV effects (Ito et al., 2004).
Table 8. $^1$H- and $^{13}$C-NMR data of compound 5 (400 MHz, in acetone-$d_6$) and (-)pauciflorol E (300 MHz, in acetone-$d_6$)

<table>
<thead>
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<th>Position</th>
<th>Compound 5</th>
<th>(-)-pauciflorol E *</th>
<th>HMBC correlation with</th>
</tr>
</thead>
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<tr>
<td></td>
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<td>$\delta_c$ (mult., $J$ in Hz)</td>
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</tr>
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<td>6.77 (dd, 8.5, 1.1)</td>
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<td>6.70 (d, 8.5)</td>
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<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>5a</td>
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<td>116.3</td>
<td>6.70 (d, 8.5)</td>
</tr>
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<td>128.4</td>
<td>6.77 (dd, 8.5, 1.1)</td>
</tr>
<tr>
<td>7a</td>
<td>6.05 (brs)</td>
<td>55.2</td>
<td>6.06 (brs)</td>
</tr>
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</tr>
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</tr>
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<td>158.4</td>
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<td>116.2</td>
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<td>6b</td>
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<td>129.9</td>
<td>7.19 (d, 8.5)</td>
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<td>11b</td>
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<td>158.9</td>
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<tr>
<td>12b</td>
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<td>13b</td>
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<td>-</td>
</tr>
<tr>
<td>Position</td>
<td>Compound 5</td>
<td>( \delta_H ) (mult., J in Hz)</td>
<td>( \delta_C )</td>
</tr>
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</tr>
<tr>
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<td>6.39 (br s)</td>
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</tr>
<tr>
<td>4b-OH</td>
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<td>-</td>
<td>8.42 (br s)</td>
</tr>
<tr>
<td>11-OH</td>
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<tr>
<td>13b-OH</td>
<td>-</td>
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<td>8.57 (br s)</td>
</tr>
</tbody>
</table>


**Figure 45.** HR-ESI mass spectrum of compound 5
Figure 46. IR spectrum of compound 5

Figure 47. $^1$H-NMR spectrum of compound 5 (400 MHz, acetone-$d_6$)
Figure 48. $^1$H-NMR spectrum of compound 5 (expansion between $\delta_H$ 4.4-7.5 ppm)

Figure 49. $^1$H-NMR spectrum of compound 5 (expansion between $\delta_H$ 4.4-7.4 ppm)
Figure 50. $^{13}$C-NMR spectrum of compound 5 (100 MHz, acetone-$d_{6}$)

Figure 51. $^{13}$C-NMR spectrum of compound 5 (expansion between $\delta_C$ 40-160 ppm)
Figure 52. $^1$H-$^{13}$C HSQC spectrum of compound 5

Figure 53. $^1$H-$^{13}$C HMBC spectrum of compound 5
(expansion between $\delta_H$ 4.2-8.0 ppm, $\delta_C$ 50-200 ppm)
Figure 54. $^1$H-$^{13}$C HMBC spectrum of compound 5
(expansion between $\delta_H$ 4.5-7.6 ppm, $\delta_C$ 90-150 ppm)

Figure 55. $^1$H-$^1$H COSY spectrum of compound 5
4.2 Structure elucidation of compounds isolated from *Maerua siamensis* roots

4.2.1 Structure elucidation of compound 6 [(+)-(maeruanitrile A)]

Compound 6 was obtained as a reddish-brown amorphous solid with a $[\alpha]_{D}^{25}$ value of +3.0° (c 0.001, MeOH). Its high-resolution ESI mass spectrum (Figure 58) displayed a sodium-adduct pseudo-molecular [M+Na]$^+$ ion peak at $m/z$ 241.0585 (calculated for $C_{11}H_{10}N_2O_3Na$, 241.0584), corresponding to a molecular formula of $C_{11}H_{10}N_2O_3$ with eight degrees of unsaturation. The IR spectrum of this compound (Figure 59) exhibited an absorption band of nitrile group at 2256 cm$^{-1}$, hydroxyl and amide N-H bands at 3291 cm$^{-1}$, aromatic ring at 1630 and 1463 cm$^{-1}$, and $\gamma$-lactam carbonyl at 1722 cm$^{-1}$. UV absorption peaks (Figure 57) were observed at $\lambda_{max}$ 218, 268 and 322 nm. These data are characteristic of oxindole moiety (Kinashi *et al.*, 1976).
The $^1$H-NMR spectrum of compound 6 (Table 9 and Figure 61) showed resonances of an ABX system of a benzene ring at $\delta_{H}$ 7.48 (1H, $d, J = 8.4$ Hz, H-4), 6.63 (1H, $dd, J = 8.4, 2.4$ Hz, H-5) and 6.53 (1H, $d, J = 2.4$ Hz, H-7), methylene protons at $\delta_{H}$ 3.09 (1H, $d, J = 16.8$ Hz, H-8a) and 2.89 (1H, $d, J = 16.8$ Hz, H-8b), a methoxy group at $\delta_{H}$ 3.81 (3H, s, 6-OCH$_3$) and a hydroxyl group at $\delta_{H}$ 5.44 ppm (1H, s).

Its $^{13}$C-NMR spectrum (Table 9 and Figure 62) showed eleven carbon resonances including those of an amide carbonyl carbon at $\delta_{C}$ 178.2 (C-2), a methoxy carbon at $\delta_{C}$ 56.5 (6-OCH$_3$), a methylene carbon at $\delta_{C}$ 28.0 (C-8), a nitrile carbon at $\delta_{C}$ 117.7 (C-9), three aromatic methine carbons at $\delta_{C}$ 98.8 (C-7), 108.4 (C-4) and 126.9 (C-4), an aliphatic quaternary carbon at $\delta_{C}$ 73.8 (C-3) and three aromatic quaternary carbons at $\delta_{C}$ 122.8 (C-3a), 144.7 (C-7a) and 163.4 (C-6).

The positions of aromatic protons on the benzene ring of this oxindole molecule were confirmed by two-dimensional NMR experiments. Long-range $^1$H-$^{13}$C HMBC correlations (Figure 64-65) between H-4 signal ($\delta_{H}$ 7.48) and the aliphatic quaternary carbon at $\delta_{C}$ 73.8 (C-3) and the downfield aromatic methine carbon at $\delta_{C}$ 163.4 ppm (C-6), whereas H-7 signal showed three-bond HMBC cross peaks with those of C-3a ($\delta_{C}$ 122.8) and C-5 ($\delta_{C}$ 108.4). The assignment of a methoxy group at position 6 of the indole ring was supported by an observed HMBC cross peak between 6-OCH$_3$ signal and C-6, as well as $^1$H-$^1$H NOESY correlations between this methoxy protons and both H-5 and H-7 signals (Figure 67). An acetonitrile group could be located at C-3, based on HMBC cross peaks of its methylene protons (H-8) with C-2 ($\delta_{C}$ 178.2), C-3 and C-3a. A hydroxyl group could also be located at C-3 based on its downfield shift and HMBC correlations from 3-OH signal (at $\delta_{H}$ 5.44 ppm) to C-3 and C-3a. The configuration at position 3 was confirmed by comparison of its ECD spectra (Figure 60) with that of a known compound, (++)-(S)-2-(3-hydroxy-4-methoxy-2-oxindolin-3-yl) acetonitrile, from Isatis indigotica (Chen et al., 2012). Therefore, the chemical structure of compound 6 was established as (++)-(S)-2-(3-hydroxy-6-methoxy-2-oxindolin-3-yl) acetonitrile, and was named (++)-maeruanitrile A.
(+)-maeruanitrile A.

Table 9. $^1$H-, $^{13}$C-NMR and HMBC data of compound 6 (400 MHz, acetone-$d_6$)

<table>
<thead>
<tr>
<th>Position</th>
<th>$\delta_\text{H}$ (mult., $J$ in Hz)</th>
<th>$\delta_\text{C}$</th>
<th>HMBC correlation with</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH-1</td>
<td>9.52, br s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>178.2</td>
<td></td>
</tr>
<tr>
<td>3</td>
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<td>73.8</td>
<td></td>
</tr>
<tr>
<td>3a</td>
<td>-</td>
<td>122.8</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>7.48 (d, 8.4)</td>
<td>126.9</td>
<td>C-3, C-6</td>
</tr>
<tr>
<td>5</td>
<td>6.63 (dd, 8.4, 2.4)</td>
<td>108.4</td>
<td>C-3a, C-6, C-7</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>163.4</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>6.53 (d, 2.4)</td>
<td>98.8</td>
<td>C-3a, C-5, C-6, C-7a</td>
</tr>
<tr>
<td>7a</td>
<td></td>
<td>144.7</td>
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</tr>
<tr>
<td>8a</td>
<td>3.09 (d, 16.8)</td>
<td>28.0</td>
<td>C-2, C-3, C-3a, C-9</td>
</tr>
<tr>
<td>8b</td>
<td>2.89 (d, 16.8)</td>
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<td></td>
</tr>
<tr>
<td>9-CN</td>
<td>-</td>
<td>117.8</td>
<td></td>
</tr>
<tr>
<td>3-OH</td>
<td>5.44, (s)</td>
<td></td>
<td>C-3, C-3a</td>
</tr>
<tr>
<td>6-OCH$_3$</td>
<td>3.81, (s)</td>
<td>56.5</td>
<td>C-6</td>
</tr>
</tbody>
</table>
Figure 57. UV spectrum of compound 6

Figure 58. HR-ESI mass spectrum of compound 6
Figure 59. IR spectrum of compound 6

Figure 60. The CD spectrum in MeOH of compound 1 (upper left) and The ECD spectrum of compound 1; calculated for $R$ configuration (lower left), calculated for $S$ configuration (lower right) and overlayed ECD spectrum (upper right).
Figure 61. $^1$H-NMR spectrum of compound 6 (600 MHz, acetone-$d_6$)

Figure 62. $^{13}$C-NMR spectrum of compound 6 (150 MHz, acetone-$d_6$)
Figure 63. $^{13}$C-NMR, DEPT135 and DEPT90 spectrum of compound 6

Figure 64. $^1$H-$^{13}$C HSQC spectrum of compound 6
Figure 65. $^1$H-$^{13}$C HMBC spectrum of compound 6

Figure 66. $^1$H-$^1$H COSY spectrum of compound 6
4.2.2 Structure elucidation of compound 7 (maeruanitrile B)

Compound 7 was obtained as a reddish-brown amorphous solid. Its molecular formula was deduced as C_{12}H_{12}N_{2}O_{2}S (nine degrees of unsaturation), based on the sodium-adduct pseudo-molecular [M+Na]^+ ion at m/z 271.0511 (calculated for C_{12}H_{12}N_{2}O_{2}SNa, 271.0512) in the HR-ESI mass spectrum (Figure 69). Its IR spectrum (Figure 70) showed strong absorption peaks of sulfoxide at 1022 cm\(^{-1}\), nitrile group at 2250 cm\(^{-1}\) hydroxyl and amine groups at 3163 cm\(^{-1}\) and aromatic ring at 1626 and 1451 cm\(^{-1}\). The UV spectrum (Figure 68) exhibited absorption maxima at \(\lambda_{\text{max}}\) 228, 300 and 342 nm.

Its \(^1\text{H}-\text{NMR data (Table 10 and Figure 71)}\) showed ABX coupling protons of a benzene ring at \(\delta_H\) 7.63 (1H, \(d, J = 9.0\) Hz, H-4), 6.98 (1H, \(d, J = 2.4\) Hz, H-7) and 6.87 (1H, \(dd, J = 9.0, 2.4\) Hz, H-5), methylene protons of an acetonitrile group at \(\delta_H\) 4.18 (1H, \(d, J = 18.0\) Hz, H-8a) and 4.13 (1H, \(d, J = 16.0\) Hz, H-8b), a methoxy singlet at \(\delta_H\) 3.86 (3H, 6-OCH\(_3\)) and a methylsulfinyl singlet at \(\delta_H\) 2.16 (3H, 2-SOCH\(_3\)).
Twelve signals were observed in the $^{13}$C-NMR spectrum of this compound (Table 10 and Figure 72). These were signals of six quaternary carbons of the indole nucleus at $\delta_c$ 160.6 (C-6), 140.3 (C-7a), 132.5 (C-2), 121.4 (C-3a), 110.8 (C-3) and 118.9 (C-9), three aromatic methines at $\delta_c$ 121.4 (C-4), 113.4 (C-5) and 95.3 (C-7), one methylene carbon of an acetonitrile group at $\delta_c$ 13.0 (C-8), a methoxy carbon at $\delta_c$ 55.9 (6-OCH$_3$) and a methylsulfoxide carbon at $\delta_c$ 40.4 (2-SOCH$_3$).

The substitution pattern on aromatic ring of this indole derivative is similar to that of compound 6, as confirmed by long-range $^1$H-$^{13}$C HMBC correlations (Figure 76-77) between H-4 signal ($\delta_h$ 7.63) with C-3 ($\delta_c$ 110.8), C-6 ($\delta_c$ 160.6) and C-7a ($\delta_c$ 140.3), as well as from H-7 signal (at $\delta_h$ 6.98) to those of C-3a ($\delta_c$ 121.4) and C-5 ($\delta_c$ 113.4). In addition, correlations between both H-5 and H-7 signals to that of 6-methoxy protons could also be observed in its $^1$H-$^1$H NOESY spectrum (Figure 79). An acetonitrile group could be located at position 3 based on HMBC cross peaks of its methylene protons ($\delta_h$ 4.18 and 4.13) with C-2 ($\delta_c$ 132.5), C-3 ($\delta_c$ 110.8) and C-3a signals ($\delta_c$ 121.4). Finally, a methylsulfoxide group could be attached at C-2, which was confirmed by a HMBC cross peak of its methyl signal (at $\delta_h$ 2.16) to this carbon signal.

These spectroscopic data indicated that compound 7 was similar to indole-3-acetonitrile, isolated from fruits of Capparis spinosa (Calis et al. 1999), except the presence of an additional methoxy group at C-6 of this new compound. A glycoside with similar indole nucleus, indole-3-acetonitrile-2-S-$\beta$-glucopyranoside, has been isolated from the roots of Isatis indigotica (Yang et al., 2014) of family Brassicaceae, which is closely related taxonomically to family Capparaceae. Thus, the chemical structure of compound 7 was established as 2-(6-methoxy-2-(methylsulfinyl)-1H-indol-3-yl) acetonitrile, and it was trivially named as maeruanitrile B.
Table 10. $^1$H-, $^{13}$C NMR and HBMC data of compound 7 (400 MHz, CD$_3$OD)

<table>
<thead>
<tr>
<th>Position</th>
<th>$\delta_{\text{H}}$ (mult., $J$ in Hz)</th>
<th>$\delta_{\text{C}}$</th>
<th>HMBC correlation with</th>
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Figure 68. UV spectrum of compound 7

Figure 69. HR-ESI mass spectrum of compound 7
Figure 70. IR spectrum of compound 7

Figure 71. $^1$H-NMR spectrum of compound 7 (400 MHz, CD$_3$OD)
Figure 72. $^{13}$C-NMR spectrum of compound 7 (100 MHz, CD$_3$OD)

Figure 73. $^{13}$C-NMR spectrum of compound 7 (expansion between δ$_c$ 111-141 ppm)
Figure 74. $^{13}$C-NMR, DEPT 135 and DEPT 90 (150 MHz, CD$_3$OD) spectra of compound 7.

Figure 75. $^1$H-$^{13}$C HSQC spectrum of compound 7.
Figure 76. $^1$H-$^{13}$C HMBC spectrum of compound 7

Figure 77. $^1$H-$^{13}$C HMBC spectrum of compound 7

(expansion between $\delta_H$ 3.4-7.5 ppm, $\delta_C$ 111-141 ppm)
Figure 78. $^1\text{H}^1\text{H}$ COSY spectrum of compound 7

Figure 79. $^1\text{H}^1\text{H}$ NOESY spectrum of compound 7
4.2.3 Structure elucidation of compound 8 (maeroxime A)

Compound 8 was obtained as a reddish-brown amorphous solid. The molecular formula was determined as C_{13}H_{18}N_{2}O_{2}S based on a pseudo-molecular [M+H]^+ ion peak observed in its HR-ESI mass spectrum (Figure 81) at m/z 265.0999 (calculated for C_{13}H_{18}N_{2}O_{2}S, 265.1005). This molecular formula suggested a molecule with seven degrees of unsaturation. Its IR spectrum (Figure 82) showed absorption bands due to hydroxyl and amine (3370 cm\(^{-1}\)), O-methyloxime and aromatic ring (1628, 1579 and 1457 cm\(^{-1}\)). UV absorption maxima of compound 8 were detected at \(\lambda_{\text{max}}\) 212, 217, 225 and 269 nm (Figure 80).

Its \(^1\)H-NMR data (Table 11 and Figure 83-84) showed an ABX aromatic proton signals, similar to previously discussed indole derivatives at \(\delta_\text{H} 7.33 (1H, d, J = 8.8\ \text{Hz},\ H-4), 6.84 (1H, d, J = 2.4\ \text{Hz},\ H-7)\) and 6.64 (1H, \text{dd}, \(J = 8.8, 2.4\ \text{Hz},\ H-5\)), and also one methoxy proton signal at \(\delta_\text{H} 3.74 (3H, s, 6-OCH_3)\). In addition, the \(^1\)H NMR spectrum showed resonances of one olefinic methine at \(\delta_\text{H} 7.03 (1H, d, J = 2.0\ \text{Hz},\ H-2)\), one aliphatic methylene at \(\delta_\text{H} 3.80 (2H, s, H-8)\), another methoxy signal at \(\delta_\text{H} 3.86 (3H, s, N-OCH_3)\), one methylthio signal at \(\delta_\text{H} 2.17 (3H, s, SCH_3)\) and one NH broad singlet at \(\delta_\text{H} 10.72\).

In the \(^{13}\)C-NMR spectrum of compound 8 (Table 11 and Figure 85), thirteen carbon signals were observed. They were those of four aromatic and olefinic methines at \(\delta_\text{C} 123.1 (C-2), 118.9 (C-4), 108.8 (C-5)\) and 94.5 (C-7), five quaternary carbons at \(\delta_\text{C} 107.8 (C-3), 121.5 (C-3a), 155.6 (C-6), 136.8 (C-7a)\) and 157.8 (C-9), one aliphatic methylene at \(\delta_\text{C} 25.9 (C-8)\), two methoxy carbons at \(\delta_\text{C} 61.5 (6-OCH_3)\) and 55.2 (N-OCH_3), and one methylthio carbon at \(\delta_\text{C} 12.5\).

These NMR data suggested compound 8 could be a 3,6-disubstituted 1H-indole derivative, based on a \(^1\)H-\(^1\)H COSY cross peak (Figure 90) between signals of NH-1 proton (\(\delta_\text{H} 10.72)\) and H-2 (\(\delta_\text{H} 7.03)\), as well as \(^1\)H-\(^1\)H NOESY correlations (Figure 91-92) between 6-OCH_3 signal with both H-5 (\(\delta_\text{H} 6.64)\) and H-7 (\(\delta_\text{H} 6.84)\). The indole nucleus is equal to six degrees of unsaturation, hence there should be one double bond in the side chain. A methylene carbon could be connected to position 3, based on \(^1\)H-\(^{13}\)C
HMBC cross peaks from proton signal at $\delta_H$ 3.80 to C-2 ($\delta_C$ 123.1), C-3 ($\delta_C$ 107.8) and C-3a ($\delta_C$ 121.5). The methylene group in the side chain also connected to a quaternary C-9, as evidenced by HMBC correlation from H$_2$-8 signal to C-9 ($\delta_C$ 157.8). The methylthio group could also be located at this olefinic carbon of an imine bond, based on three-bond HMBC correlation observed from its proton signal ($\delta_H$ 2.17) to C-9. Finally, the N-OCH$_3$ group was placed at the other end of this side chain, completing a methyl-N-methoxyethanimidothioate-2-yl substitution at C-3 of the indole nucleus. The NOESY cross peaks were observed between H-8 signal to both H-2 and H-4. The cis orientation between the N-OCH$_3$ and SCH$_3$ groups was suggested by the most stable conformer due to the lowest relative energy (0.00 kcal/mol) based on a DFT calculation at a B3LYP/6-31g (d,p) level (Figure 93). Thus, the structure of compound 8 was elucidated as methyl (Z)-N-methoxy-2-(6-methoxy-1H-indol-3-yl) ethanimidothioate and trivially named as maeroxime A.
Table 11. $^1$H-, $^{13}$C-NMR and HMBC data of compound 8 (400 MHz, DMSO-$d_6$)

<table>
<thead>
<tr>
<th>Position</th>
<th>$\delta_H$ (mult., $J$ in Hz)</th>
<th>$\delta_C$</th>
<th>HMBC correlation with</th>
</tr>
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<tbody>
<tr>
<td>NH-1</td>
<td>10.72 (br s)</td>
<td>-</td>
<td>C-2, C-3, C-3a, C-7a</td>
</tr>
<tr>
<td>2</td>
<td>7.03 (d, 2.0)</td>
<td>123.1</td>
<td>C-3, C-3a, C-7a, C-8</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>107.8</td>
<td>-</td>
</tr>
<tr>
<td>3a</td>
<td>-</td>
<td>121.5</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>7.33 (d, 8.8)</td>
<td>118.9</td>
<td>C-3, C-3a, C-6, C-7a</td>
</tr>
<tr>
<td>5</td>
<td>6.64 (dd, 8.8, 2.4)</td>
<td>108.8</td>
<td>C-3a, C-6, C-7</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>155.6</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>6.84 (d, 2.4)</td>
<td>94.5</td>
<td>C-3a, C-5, C-6, C-7a</td>
</tr>
<tr>
<td>7a</td>
<td>-</td>
<td>136.8</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>3.80 (s)</td>
<td>25.9</td>
<td>C-2, C-3, C-3a, C-9</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>157.8</td>
<td>-</td>
</tr>
<tr>
<td>6-OCH$_3$</td>
<td>3.74 (s)</td>
<td>55.2</td>
<td>C-6</td>
</tr>
<tr>
<td>SCH$_3$</td>
<td>2.17 (s)</td>
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<tr>
<td>N-OCH$_3$</td>
<td>3.85 (s)</td>
<td>61.5</td>
<td>-</td>
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</tbody>
</table>
Figure 80. UV spectrum of compound 8

Figure 81. HR-ESI mass spectrum of compound 8
Figure 82. IR spectrum of compound 8

Figure 83. $^1$H-NMR spectrum of compound 8 (400 MHz, DMSO-d$_6$)
Figure 84. $^1$H-NMR spectrum of compound 8 (expansion between $\delta_H 6.4-7.5$ ppm)

Figure 85. $^{13}$C-NMR spectrum of compound 8 (400 MHz, DMSO-$d_6$)
Figure 86. $^{13}$C-NMR, DEPT 135 and DEPT 90 spectrum of compound 8

Figure 87. $^1$H-$^{13}$C HSQC spectrum of compound 8
Figure 88. $^1$H-$^{13}$C HMBC spectrum of compound 8

Figure 89. $^1$H-$^{13}$C HMBC spectrum of compound 8
(expansion between $\delta_4$ 1.8-4.4 ppm, $\delta_c$ 90-180 ppm)
Figure 90. $^1$H-$^1$H COSY spectrum of compound 8

Figure 91. $^1$H-$^1$H NOESY spectrum of compound 8
Figure 92. $^1$H-$^1$H NOESY spectrum of compound 8
(expansion between $\delta_H$ 3.5-10.5 ppm)

Figure 93. Possible conformations of compound 8, based on the DFT calculation at B3LYP/6-31g (d, p) level
4.2.4 Structure elucidation of compound 9 (maeroxime B)

Compound 9 was obtained as a orange-brown amorphous solid. Its molecular formula was established as C\textsubscript{13}H\textsubscript{14}N\textsubscript{2}O\textsubscript{5}S, according to the [M+H]\textsuperscript{+} ion at \textit{m/z} 279.0782 (Calculated for C\textsubscript{13}H\textsubscript{14}N\textsubscript{2}O\textsubscript{5}S, 279.0803) (Figure 96), revealing more than 14 mass units compared to that of compound 7. Its IR absorption peaks IR (Figure 95) were similar to those of compounds 8 except a carbonyl peak at 1617 cm\textsuperscript{-1}. UV absorption were detected at \(\lambda_{\text{max}}\) 212, 280 and 314 nm (Figure 94).

The \(^1\text{H}-\text{NMR}\) data (Table 12, Figure 97) showed ABX coupled protons at \(\delta_H\) 7.92 (1H, \(d, J=8.4\) Hz, H-4), 7.00 (1H, s, H-7) and 6.89 (1H, \(d, J=8.4\) Hz, H-5), one methine proton at \(\delta_H\) 7.97 (1H, s, H-2), two methoxy groups at \(\delta_H\) 3.79 (3H, s, 6-OCH\textsubscript{3}) and 3.73 (3H, s, N-OCH\textsubscript{2}), one methylthio signal at \(\delta_H\) 2.43 (3H, s, SCH\textsubscript{3}) and one NH proton at \(\delta_H\) 12.10 (1H, br s, NH-1).

In \(^1\text{C}-\text{NMR}\) spectrum (Figure 98), thirteen signals represented four methines [\(\delta_C\) 136.7 (C-2), 121.4 (C-4), 112.3 (C-5) and 95.8 (C-7)], six quaternary carbons [\(\delta_C\) 113.6 (C-3), 118.5 (C-3a), 156.9 (C-6), 138.0 (C-7a), 182.5 (C-8), 155.9 (C-9)] and three methyl carbons [\(\delta_C\) 55.3 (6-OCH\textsubscript{3}), 61.9 (N-OCH\textsubscript{2}) and 12.7 (SCH\textsubscript{3})]. Together with \(^1\text{H}-^1\text{H}\) NOESY cross peaks of NH-1 with H-2 and H-7, the \(^1\text{H-}\) and \(^1\text{C}-\text{NMR}\) data indicated the structure of compound 9 could be 3,6-disubstituted 1H-indole ring as similar with compound 8, except the presence of carbonyl carbon at \(\delta_C\) 182.5 (C-8). In addition, \(^1\text{H-}\text{HMBC}\) cross peaks of H-2 to C-3 and C-8, and of SCH\textsubscript{3} to C-9 supported the substitution of 2-oxoethanimidothioate at C-3.

The computational studies suggested that N-methoxy was connected with imine bond as cis direction regarding to SCH\textsubscript{3} due to its lowest energy conformation of compound 9 (Figure 104). Thus, the compound 9 was established as methyl (Z)-N-methoxy-2-(6-methoxy-1H-indol-3-yl)-2-oxoethanimidothioate and was named as maeroxime B.
**Table 12.** $^1$H-, $^{13}$C-NMR and HMBC data of compound 9 (400 MHz, DMSO-$d_6$)

<table>
<thead>
<tr>
<th>Position</th>
<th>$\delta_H$ (mult., $J$ in Hz)</th>
<th>$\delta_C$</th>
<th>HMBC correlation with</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH-1</td>
<td>12.10, (br s)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>7.97, (s)</td>
<td>136.7</td>
<td>C-3, C-3a, C-7a, C-8</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>113.6</td>
<td>-</td>
</tr>
<tr>
<td>3a</td>
<td>-</td>
<td>118.5</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>7.92 (d, 8.4)</td>
<td>121.4</td>
<td>C-3, C-6, C-7a</td>
</tr>
<tr>
<td>5</td>
<td>6.89 (d, 8.4)</td>
<td>112.3</td>
<td>C-3a, C-6, C-7</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>156.9</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>7.00, (s)</td>
<td>95.8</td>
<td>C-3a, C-5, C-6, C-7a</td>
</tr>
<tr>
<td>7a</td>
<td>-</td>
<td>138.0</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>182.5</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>155.9</td>
<td>-</td>
</tr>
<tr>
<td>6-OCH$_3$</td>
<td>3.79, (s)</td>
<td>55.3</td>
<td>C-6</td>
</tr>
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<td>SCH$_3$</td>
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<tr>
<td>N-OCH$_3$</td>
<td>3.73, (s)</td>
<td>61.9</td>
<td>-</td>
</tr>
</tbody>
</table>
Figure 94. UV spectrum of compound 9

Figure 95. IR spectrum of compound 9
Figure 96. HR-ESI mass spectrum of compound 9

Figure 97. $^1$H-NMR spectrum of compound 9 (100 MHz, DMSO-d$_6$)
Figure 98. $^{13}$C-NMR spectrum of compound 9 (100 MHz, DMSO-$d_6$)

Figure 99. $^1$H-$^{13}$C HSQC spectrum of compound 9
Figure 100. $^1$H-\(^{13}\)C HMBC spectrum of compound 9
(expansion between $\delta_H$ 2.0-8.4 ppm, $\delta_C$ 100-180 ppm)

Figure 101. $^1$H-\(^{13}\)C HMBC spectrum of compound 9
Figure 102. $^1$H-$^1$H COSY spectrum of compound 9

Figure 103. $^1$H-$^1$H NOESY spectrum of compound 9
Figure 104. Possible conformations of compound 9, based on the DFT calculation at B3LYP/6-31g (d, p) level

4.2.5 Structure elucidation of compound 10 (maeroxime C)

Compound 10 was obtained as a yellow amorphous solid. Its molecular formula was deduced as C_{13}H_{15}N_{2}O_{3}S (eight degrees of unsaturation), based on a pseudo-molecular [M+H]^+ ion peak observed at m/z 279.0780 (calculated for C_{13}H_{15}N_{2}O_{3}S, 279.0803) in the HR-ESI mass spectrum (Figure 106). Therefore, this compound was a structural isomer of compound 9. Its major IR absorption bands (Figure 107) were observed at 3307 and 1729 cm\(^{-1}\) and UV absorption maxima (Figure 105) were detected at \(\lambda_{\text{max}}\) 208, 272 and 316 nm. These data are characteristic of oxindole moiety, similar to compound 6.

The \(^1\)H-NMR data of compound 10 (Table 13 and Figure 108) showed ABX proton system at \(\delta_H\) 7.87 (1H, d, \(J = 8.4\) Hz, H-4), 6.41 (1H, d, \(J = 2.4\) Hz, H-7) and 6.52 (1H, dd, \(J = 8.4, 2.4\) Hz, H-5), one olefinic methine proton at \(\delta_H\) 6.80 (1H, s, H-8), two methoxy protons at \(\delta_H\) 3.78 (3H, s, 6-\text{OCH}_3) and 4.00 (3H, s, N-\text{OCH}_3), one methylthio signal at \(\delta_H\) 2.35 (3H, s, S\text{CH}_3) and one NH broad singlet at \(\delta_H\) 10.63.

Its \(^{13}\)C-NMR spectrum (Table 13 and Figure 109) displayed thirteen carbon resonances representing an amide carbonyl at \(\delta_C\) 168.6 (C-2), four aromatic and olefinic
methines at $\delta_C$ 126.5 (C-4), 106.8 (C-5), 96.6 (C-7) and 119.9 (C-8), five quaternary carbons at $\delta_C$ 131.1 (C-3), 113.3 (C-3a), 162.2 (C-6), 145.6 (C-7a) and 150.8 (C-9), two methoxy carbons at $\delta_C$ 55.5 (6-OCH$_3$) and 62.5 (N-OCH$_3$), and one methylthio carbon at $\delta_C$ 12.6 (SCH$_3$).

Its oxindole characteristic indicated that position 2 of this 3,6-disubstituted 1H-oxindole nucleus should be the amide carbonyl function, hence a double bond was placed to between positions 3 and 8. This was confirmed by $^1$H-$^1$C HMBC correlations (Figure 111) observed from H-8 to C-2, C-3, C-3a and C-9. Other substituents were located the same positions as compound 9. The trans, trans geometric isomer was introduced by the $^1$H-$^1$H NOESY cross peaks of H-4 and N-OCH$_3$, and of H-8 and SCH$_3$, together with the lowest energy data from computational analysis (Figure 113 and 114). Therefore, the chemical structure of compound 10 was determined as (E)-N-methoxy-2-((E)-6-methoxy-2-oxindolin-3-ylidene) ethanimidothiolate and trivially named maeroxime C.
Table 13. $^1$H-, $^{13}$C-NMR and HMBC data of compound 10 (400 MHz, DMSO-$d_6$)

<table>
<thead>
<tr>
<th>Position</th>
<th>$\delta_H$ (mult., $J$ in Hz)</th>
<th>$\delta_C$</th>
<th>HMBC correlation with</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH-1</td>
<td>10.63 (br s)</td>
<td>-</td>
<td>C-2, C-3, C-3a, C-7a</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>168.6</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>131.1</td>
<td>-</td>
</tr>
<tr>
<td>3a</td>
<td>-</td>
<td>113.3</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>7.87 (d, 8.4)</td>
<td>126.5</td>
<td>C-3, C-5, C-6, C-7a</td>
</tr>
<tr>
<td>5</td>
<td>6.52 (dd, 8.4, 2.4)</td>
<td>106.8</td>
<td>C-3a, C-6, C-7</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>7</td>
<td>6.41 (d, 2.4)</td>
<td>96.6</td>
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<td>150.8</td>
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<tr>
<td>6-OCH$_3$</td>
<td>3.78 (s)</td>
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<td>N-OCH$_3$</td>
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Figure 105. UV spectrum of compound 10
Figure 106. HR-ESI mass spectrum of compound 10

Figure 107. IR spectrum of compound 10
Figure 108. $^1$H-NMR spectrum of compound 10 (400 MHz, DMSO-$_d_6$)

Figure 109. $^{13}$C-NMR spectrum of compound 10 (100 MHz, DMSO-$_d_6$)
Figure 110. $^1\text{H}-^{13}\text{C}$ HSQC spectrum of compound 10

Figure 111. $^1\text{H}-^{13}\text{C}$ HMBC spectrum of compound 10
Figure 112. $^1$H-$^1$H COSY spectrum of compound 10

Figure 113. $^1$H-$^1$H NOESY spectrum of compound 10
Figure 114: Possible conformations of compound 10, based on the DFT calculation at B3LYP/6-31g (d, p) level.

4.2.6 Structure elucidation of compound 11 (maeruabisindole A)

Compound 11 was isolated as a pale green amorphous solid, showing UV absorption maxima at \( \lambda_{\text{max}} \) 210, 270, 315, 355 and 365 nm (Figure 115). The HR-ESI mass spectrum (Figure 116) exhibited a pseudo-molecular [M+H]+ ion peak at \( m/z \) 390.1298, corresponding to a molecular formula of C_{22}H_{19}N_{3}O_{2}S (calculated for C_{22}H_{20}N_{3}O_{2}S, 390.1271), indicating fifteen degrees of unsaturation. Its IR spectrum (Figure 117) showed absorption bands of amine and imine at 3384 and 1625 cm\(^{-1}\), respectively.

The \(^1\)H-NMR (400 MHz, DMSO-\(d_6\)) data of compound 11 (Table 14 and Figure 118-119) displayed resonances of two set of disubstituted-\(^1\)H-indole moieties at \( \delta_{\text{H}} \) 10.71 (1H, br s, NH-1'), 8.05 (1H, d, \( J = 8.4 \) Hz, H-4'), 7.79 (1H, br s, H-3'), 6.99 (1H, d, \( J = 2.4 \) Hz, H-7') and 6.78 (1H, \( dd, J = 8.4, 2.0 \) Hz, H-5') and at \( \delta_{\text{H}} \) 11.52 (1H, br s, NH-1''),
7.51 (1H, d, J = 2.0 Hz, H-2'), 7.12 (1H, overlapped, H-7'''), 7.10 (1H, overlapped, H-6'''') and 6.56 (1H, dd, J = 6.0, 2.4 Hz, H-5'''). Two methoxy signals were observed at δH 3.81 (3H, s, 6'-OCH3) and 3.52 (3H, s, 4'''-OCH3), while a methylthio signal could be seen at δH 2.59 (3H, s, 2-SCH3). Two indole nuclei, representing sixteen carbon atoms and twelve degrees of unsaturation, indicated that compound 11 was a bisindole alkaloid. Disregarding two methoxy and one methylthio groups, the last part of this molecule should involve one nitrogen atom and three carbon atoms in an imine formation with three degrees of unsaturation.

Its 13C-NMR (400 MHz, DMSO-d6) data (Table 14 and Figure 120-121) exhibited 22 signals representing two methoxy groups at δC 55.2 (6'-OCH3) and 54.9 (4'''-OCH3), one methylthio group at δC 140.0 (2-SCH3), methine and quaternary carbons of two indole moieties at δC 160.0 (C, C-6'), 153.9 (C, C-4''), 142.9 (C, C-7'a), 137.9 (C, C-7''a), 129.3 (C, C-2'), 125.0 (CH, C-2''), 122.6 (CH, C-4''), 122.5 (CH, C-6''), 116.4 (C, C-3''a), 114.1 (C, C-3'a), 113.0 (C, C-3''), 108.7 (CH, C-5'), 108.5 (CH, C-3'), 105.0 (CH, C-7''), 100.4 (CH, C-5''') and 94.8 (CH, C-7'), and three sp2 carbons of a 2,3,4-trisubstituted azete moiety (Csaszar et al., 2015) at δC 144.8 (C-2), 139.5 (C-4) and 133.4 (C-3).

A methoxy group could be located at position 6' of an indole moiety. This was confirmed by 1H-13C HMQC correlations (Figure 123-126) observed from its proton signal (6'-OCH3) to C-6', from NH-1' to C-2', C-3'a and C-7'a and from H-4' to C-6' and C-7'a, together with 1H-1H NOESY cross peaks (Figure 128) between proton signals of NH-1' and H-7'; of H-3' and H-4' and of 6'-OCH3 with H-5' and H-7'. Another methoxy group was assigned at position 4'' of the second indole moiety based on HMBC correlations observed from the signal of 4''-OCH3 to C-4''; from NH-1'' to C-3''a and C-7''a and from H-6'' to C-4'' and C-7''a, together with NOESY cross peaks between NH-1'' and H-7'' and between 4''-OCH3 and H-5''.

Both indole nuclei were connected through an azete ring. The 6'-methoxy indole moiety could be connected via C-2' to this four-membered ring at position 3, based on a HMBC cross peaks from NH-1' and H-3' to C-3, whereas the 4''-methoxy indole moiety was connected via C-3'' to position 4 of the azete ring, as supported by a 1H-1H COSY cross peak (Figure 127) between NH-1'' and H-2', and a
HMBC cross peak from H-2" to C-4 signal. Finally, the methylthio group was located at position 2 of the azete ring based on a HMBC correlation from its proton signal ($\delta_H$ 2.59) to C-2. Therefore, the structure of compound 11 was elucidated as 4-methoxy-3-(3-(6-methoxy-1H-indol-2-yl)-4-(methylthio)azet-2-yl)-1H-indole and was trivially named maeruabisindole A. Bisindole alkaloids bearing an azete ring have previously been found in the roots of *Isatis tinctoria* (family Brassicaceae). Two of these indole derivatives, namely isatindigosides G and F, exhibited nitric oxide inhibitory effect (Zhang et al., 2020).
Table 14. $^1$H-, $^{13}$C-NMR and HBMC data of compound 11 (400 MHz, in DMSO-d$_6$)

<table>
<thead>
<tr>
<th>Position</th>
<th>$\delta_H$ (mult., $J$ in Hz)</th>
<th>$\delta_C$</th>
<th>HMBC correlation with</th>
</tr>
</thead>
<tbody>
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<td>144.8</td>
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<td>3</td>
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</tr>
<tr>
<td>4</td>
<td>-</td>
<td>139.5</td>
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</tr>
<tr>
<td>NH-1$'$</td>
<td>10.71 (br s)</td>
<td>-</td>
<td>C-2$'$, C-3$'$a, C-7$'$a, C-3</td>
</tr>
<tr>
<td>2$'$</td>
<td>-</td>
<td>129.3</td>
<td></td>
</tr>
<tr>
<td>3$'$</td>
<td>7.79 (br s)</td>
<td>108.5</td>
<td>C-3, C-3$'$a</td>
</tr>
<tr>
<td>3$'$a</td>
<td>-</td>
<td>114.1</td>
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</tr>
<tr>
<td>4$'$</td>
<td>8.05 (d, 8.4)</td>
<td>122.6</td>
<td>C-6$'$, C-7$'$a</td>
</tr>
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<td>6.78 (dd, 8.4, 2.0)</td>
<td>108.7</td>
<td>C-3$'$a, C-7$'$</td>
</tr>
<tr>
<td>6$'$</td>
<td>-</td>
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</tr>
<tr>
<td>7$'$</td>
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<td>C-3$'$a, C-5$'$, C-6$'$, C-7$'$a</td>
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<td>7$'$a</td>
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<td></td>
</tr>
<tr>
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<tr>
<td>3$''$</td>
<td>-</td>
<td>113.0</td>
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</tr>
<tr>
<td>3$''$a</td>
<td>-</td>
<td>116.4</td>
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<tr>
<td>4$''$</td>
<td>-</td>
<td>153.9</td>
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<td>6.56 (dd, 6.0, 2.4)</td>
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<td>C-4$''$, C-7$''$a</td>
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<tr>
<td>7$''$a</td>
<td>-</td>
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<tr>
<td>2-SCH$_3$</td>
<td>2.59 (s)</td>
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<td>4$''$- OCH$_3$</td>
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Figure 115. UV spectrum of compound 11

Figure 116. HR-ESI mass spectrum of compound 11
Figure 117. IR spectrum of compound 11

Figure 118. $^1$H-NMR spectrum of compound 11 (400 MHz, DMSO-$d_6$)
Figure 119. $^1$H-NMR spectrum of compound 11 (expansion between $\delta_H$ 6.3-8.3 ppm)

Figure 120. $^{13}$C-NMR spectrum of compound 11 (100 MHz, DMSO-$d_6$)
Figure 121. $^{13}$C-NMR spectrum of compound 11 (100 MHz, DMSO-$d_6$)
(expansion between $\delta$C 90-170 ppm)

Figure 122. $^1$H-$^{13}$C HSQC spectrum of compound 11
Figure 123. $^1$H-$^{13}$C HMBC spectrum of compound 11
(expansion between $\delta_H$ 0.5-12.0 ppm, $\delta_C$ 20-180 ppm)

Figure 124. $^1$H-$^{13}$C HMBC spectrum of compound 11
(expansion between $\delta_H$ 2.0-12.0 ppm, $\delta_C$ 85-160 ppm)
Figure 125. $^1$H-$^{13}$C HMBC spectrum of compound 11
(expansion between $\delta_H 10.4-12.0$ ppm, $\delta_C 104-158$ ppm)

Figure 126. $^1$H-$^{13}$C HMBC spectrum of compound 11
(expansion between $\delta_H 6.4-8.4$ ppm, $\delta_C 90-164$ ppm)
Figure 127. $^1$H-$^1$H COSY spectrum of compound 11

Figure 128. $^1$H-$^1$H NOESY spectrum of compound 11
4.2.7 Structure elucidation of compound 12 (maeabuisindole B)

Compound 12 was obtained as a pale green amorphous solid, showing UV absorption maxima at $\lambda_{\text{max}}$ 210, 230, 310, 340 and 355 nm (Figure 129). The IR spectrum (Figure 131) showed absorption bands of amine at 3396 cm$^{-1}$, imine and aromatic ring at 1602 and 1465 cm$^{-1}$ and sulfoxide group 1025 cm$^{-1}$. Its molecular formula of C$_{22}$H$_{20}$N$_{2}$O$_{3}$S was deduced from a pseudo-molecular [M+H]$^+$ ion peak at m/z 406.1224 (calculated for C$_{22}$H$_{20}$N$_{2}$O$_{3}$S, 406.1220) in the HR-ESI mass spectrum (Figure 130).

Its $^1$H and $^{13}$C-NMR data are mostly similar to those of compound 11. The $^1$H-NMR spectrum of compound 12 (400 MHz, CD$_3$OD) (Table 15 and Figure 132-133) showed peaks of the 2',6'-disubstituted-1H-indole moiety at $\delta_{\text{H}}$ 8.12 (1H, d, $J$ = 8.4 Hz, H-4'), 8.43 (1H, s, H-3'), 7.05 (1H, d, $J$ = 2.0 Hz, H-7'), 6.93 (1H, dd, $J$ = 8.4, 2.0 Hz, H-5') and 3.89 (3H, $s$, 6'-OCH$_3$) and the 3'',4''-disubstituted-1H-indole moiety at $\delta_{\text{H}}$ 7.54 (1H, s, H-2''), 7.14 (1H, d, $J$ = 6.8 Hz, H-7''), 7.16 (1H, dd, $J$ = 7.6, 6.8 Hz, H-6''), 6.60 (1H, d, $J$ = 6.8 Hz, H-5'') and 3.55 (3H, $s$, 4''-OCH$_3$). A methylthio signal, as seen in compound 11, was replaced by a methylsulfoxide singlet at $\delta_{\text{H}}$ 2.98 (3H, $s$, 2-SOCH$_3$). The H-3' signal appeared at more downfield chemical shift due to the anisotropic effect of S=O bond of the methylsulfoxide group on the nearby azete ring to this proton on the indole nucleus.

The $^{13}$C-NMR data of this compound (400 MHz, CD$_3$OD) (Table 15 and Figure 134) showed 22 carbon peaks representing two methoxy carbons at $\delta_{\text{C}}$ 56.1 (6'-OCH$_3$) and 55.7 (4''-OCH$_3$), one methylsulfoxide carbon at $\delta_{\text{C}}$ 42.1 (2-SOCH$_3$), methine carbons at $\delta_{\text{C}}$ 126.1 (C-2''), 124.4 (C-6''),123.7 (C-4''), 111.8 (C-5''), 109.3 (C-3''), 106.3 (C-7''), 101.7 (C-5'') and 95.8 (C-7'') and quaternary carbons at $\delta_{\text{C}}$ 163.0 (C-6''), 155.6 (C-4''), 144.9 (C-7'a), 140.0 (C-7''a), 130.8 (C-2''), 118.2 (C-3''a), 116.3 (C-3'a) and 113.2 (C-3''). Three $sp^2$ carbons of the 2,3,4-trisubstituted azete ring resonated at $\delta_{\text{C}}$ 151.7 (C-2), 142.7 (C-4) and 137.8 (C-3).

These spectroscopic data indicated that the difference of this compound to compound 11 was the presence of a methylsulfoxide group, instead of a methylthio group, at position 2 of the azete ring. This was confirmed by $^1$H-$^{13}$C HMBC correlation.
between the methylsulfoxide proton signal (δH, 2.98) to C-2 of the azete ring. Linkage of the 2',6'-disubstituted indole unit to the azete ring was confirmed by HMBC cross peaks from H-3' to C-3 and C-3'a. Therefore, the structure of compound 12 was established as 4-methoxy-3-(3-(6-methoxy-1H-indol-2-yl)-4-(methysulfinyl)azet-2-yl)-1H-indole and named maeruabisindole B.
Table 15. $^1$H-, $^{13}$C NMR and HBMC data of compound 12 (400 MHz, in CD$_3$OD)

<table>
<thead>
<tr>
<th>Position</th>
<th>$\delta_H$ (mult., $J$ in Hz)</th>
<th>$\delta_C$</th>
<th>HMBC correlation with</th>
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<tbody>
<tr>
<td>2</td>
<td>-</td>
<td>151.7</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>-</td>
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</tr>
<tr>
<td>4</td>
<td>-</td>
<td>142.7</td>
<td>-</td>
</tr>
<tr>
<td>2'</td>
<td>-</td>
<td>130.8</td>
<td>-</td>
</tr>
<tr>
<td>3'</td>
<td>8.43 (s)</td>
<td>109.3</td>
<td>C-3, C-3'a</td>
</tr>
<tr>
<td>3'a</td>
<td>-</td>
<td>116.3</td>
<td>-</td>
</tr>
<tr>
<td>4'</td>
<td>8.12 (d, 8.4)</td>
<td>123.7</td>
<td>C-2', C-6', C-7'a</td>
</tr>
<tr>
<td>5'</td>
<td>6.93 (dd, 8.4, 2.0)</td>
<td>111.8</td>
<td>C-3'a, C-7'</td>
</tr>
<tr>
<td>6'</td>
<td>-</td>
<td>163.0</td>
<td>-</td>
</tr>
<tr>
<td>7'</td>
<td>7.05 (d, 2.0)</td>
<td>95.8</td>
<td>C-3'a, C-5', C-6'</td>
</tr>
<tr>
<td>7'a</td>
<td>-</td>
<td>144.9</td>
<td>-</td>
</tr>
<tr>
<td>2''</td>
<td>7.54 (s)</td>
<td>126.1</td>
<td>C-3'', C-3''a, C-7''a</td>
</tr>
<tr>
<td>5''</td>
<td>-</td>
<td>113.2</td>
<td>-</td>
</tr>
<tr>
<td>3''a</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>4''</td>
<td>-</td>
<td>155.6</td>
<td>-</td>
</tr>
<tr>
<td>5''</td>
<td>6.60 (d, 6.8)</td>
<td>101.7</td>
<td>C-3''a, C-4'', C-7''</td>
</tr>
<tr>
<td>6''</td>
<td>7.16 (dd, 7.6, 6.8)</td>
<td>124.4</td>
<td>C-5''</td>
</tr>
<tr>
<td>7''</td>
<td>7.14 (d, 6.8)</td>
<td>106.3</td>
<td>C-4'', C-5'', C-7''a</td>
</tr>
<tr>
<td>7''a</td>
<td>-</td>
<td>140.0</td>
<td>-</td>
</tr>
<tr>
<td>2-SOCH$_3$</td>
<td>2.98 (s)</td>
<td>42.1</td>
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<tr>
<td>6'-OCH$_3$</td>
<td>3.89 (s)</td>
<td>56.1</td>
<td>-</td>
</tr>
<tr>
<td>4''-OCH$_3$</td>
<td>3.55 (s)</td>
<td>55.7</td>
<td>-</td>
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</table>
Figure 129. UV spectrum of compound 12

Figure 130. HR-ESI mass of compound 12
Figure 131. IR spectrum of compound 12

Figure 132. $^1$H-NMR spectrum of compound 12 (400 MHz, CD$_3$OD)
Figure 133. $^1$H-NMR spectrum of compound 12 (expansion between $\delta_H$ 6.3-8.7 ppm)

Figure 134. $^{13}$C-NMR spectrum of compound 12 (100 MHz, CD$_3$OD)
Figure 135. $^1$H-$^{13}$C HSQC spectrum of compound 12

Figure 136. $^1$H-$^{13}$C HSQC spectrum of compound 12
(expansion between $\delta_H$ 6.0-9.1 ppm, $\delta_C$ 90-130 ppm)
Figure 137. $^1$H-$^{13}$C HMBC spectrum of compound 12
(expansion between $\delta_{H}$ 6.3-8.6 ppm, $\delta_{C}$ 90-170 ppm)

Figure 138. $^1$H-$^{13}$C HMBC spectrum of compound 12
(expansion between $\delta_{H}$ 2.1-4.4 ppm, $\delta_{C}$ 140-170 ppm)
Figure 139. $^1$H-$^1$H COSY spectrum of compound 12
(expansion between $\delta_H$ 6.0-9.0 ppm)

Figure 140. $^1$H-$^1$H NOESY spectrum of compound 12
(expansion between $\delta_H$ 2.0-9.0 ppm)
4.2.8 Structure elucidation of compound 13 (maeruabisindole C)

Compound 13 was isolated as a dark green amorphous solid, having a molecular formula of $C_{20}H_{12}N_3O_2$ based on its pseudo molecular [M-H]$^-$ ion peak in the HR-ESI mass spectrum (Figure 141) at $m/z$ 326.0968 (calculated for $C_{20}H_{12}N_3O_2$, 326.0935), requiring sixteen degrees of unsaturation. Its IR spectrum (Figure 143) showed absorption bands due to hydroxyl and amine groups (3359 cm$^{-1}$), nitrile (2212 cm$^{-1}$) and aromatic ring (1632 and 1468 cm$^{-1}$). Its UV absorption maxima were measured at $\lambda_{\text{max}}$ 210, 285, 355 and 365 nm (Figure 142).

The $^1$H NMR spectra of compound 13 (Table 16 and Figure 144-145) showed signals of one 1,2,3-trisubstituted benzene ring at $\delta_H$ 6.80 (1H, d, $J = 8.0$ Hz, H-2), 7.40 (1H, t, $J = 8.0$ Hz, H-3) and 7.24 (1H, d, $J = 8.0$ Hz, H-4), one 1,2,4-trisubstituted benzene ring at $\delta_H$ 8.33 (1H, d, $J = 8.8$ Hz, H-7), 6.86 (1H, dd, $J = 8.8$, 2.4 Hz, H-8) and 7.02 (1H, d, $J = 2.4$ Hz, H-10), an aromatic proton at $\delta_H$ 8.53 (1H, s, H-12), two NH protons at $\delta_H$ 10.86 (1H, br s, NH-5) and 10.39 (1H, br s, NH-11), a methoxy group at $\delta_H$ 4.12 (3H, s, 1-OCH$_3$) and a hydroxyl group at $\delta_H$ 8.70 (1H, br s, 9-OH).

Its $^{13}$C NMR spectra (Table 16 and Figure 146-147) showed signals of eleven quaternary carbons at $\delta_C$ 113.0 (C-12b), 115.4 (C-6b), 82.5 (C-6), 122.0 (C-12a), 123.0 (C-6a), 138.0 (C-5a), 135.8 (C-11a), 143.4 (C-4a), 144.6 (C-10a), 157.1 (C-1) and 158.8 (C-9), seven methine carbons at $\delta_C$ 97.5 (C-10), 101.5 (C-2), 105.1 (C-4), 110.0 (C-8), 110.2 (C-12), 122.6 (C-7) and 128.2 (C-3), a methoxy carbon at $\delta_C$ 56.0 (1-OCH$_3$), and a nitrile carbon at $\delta_C$ 118.3 (6-CN).

These spectroscopic data indicated that the structure of compound 13 comprised of two indole rings connected into the core structure of indolo[3,2-b]carbazole (Wahlström et al., 2007). The $^1$H-$^{13}$C HMBC cross peaks from NH-5 to C-5a, C-6, C-12a and C-12b, from NH-11 signal to C-6a, C-6b, and C-10a and from H-12 to C-5a, C-6a, C-12a and C-12b confirmed this skeleton. A methoxy group could be located at C-1 based on a HMBC correlation observed from its proton signal ($\delta_H$ 4.12) to C-1 and a $^1$H-$^1$H NOESY cross peak between its signal and that of H-2. The hydroxyl substitution at C-9 was proven by the HMBC correlations of its proton signal ($\delta_H$ 8.70).
to C-8, C-9 and C-10, as well as its $^1$H-$^1$H NOESY correlations with both H-8 and H-10. In addition, a NOESY cross peak was also observed between H-12 and NH-11. Finally, the nitrile group could be placed at position 6 of the indolo[3,2-b]carbazole nucleus. The downfield chemical shifts of H-7 and NH-5 signals might be due to the anisotropic effect of this nitrile group. Thus, the chemical structure of compound 13 was elucidated as 9-hydroxy-1-methoxy-5,11-dihydroindolo[3,2-b]carbazole-6-carbonitrile. It was given the name maeruabisindole C.
Table 16. $^1$H-, $^{13}$C NMR and HBMC data of compound 13 (400 MHz, in acetone-$d_6$)

<table>
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<tr>
<th>Position</th>
<th>Position</th>
<th>$\delta_\text{H}$ (mult., $J$ in Hz)</th>
<th>$\delta_\text{C}$</th>
<th>HMBC correlation with</th>
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<td>$\delta_\text{C}$</td>
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</tr>
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<td>6.80 (d, 8.0)</td>
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<td>7.40 (t, 8.0)</td>
<td>128.2</td>
<td>C-1, C-1a, C-2, C-4a</td>
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<td>7.24 (d, 8.0)</td>
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<td>4a</td>
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<td>-</td>
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<tr>
<td>NH-5</td>
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<td>10.86, br s</td>
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<td>C-1a, C-5a, C-12a</td>
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<td>82.5</td>
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<tr>
<td>6a</td>
<td></td>
<td>123.0</td>
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<td>-</td>
</tr>
<tr>
<td>6b</td>
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<td>115.4</td>
<td></td>
<td>-</td>
</tr>
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<td>8.33 (d, 8.8)</td>
<td>122.6</td>
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<td>6.86 (dd, 8.8, 2.4)</td>
<td>110.0</td>
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<td>9</td>
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<td>158.8</td>
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<td>7.02 (d, 2.4)</td>
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<td>10.39, br s</td>
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<td>C-6a, C-7a, C-10a</td>
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<td>11a</td>
<td></td>
<td>135.8</td>
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<td>-</td>
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<tr>
<td>12</td>
<td></td>
<td>8.53, s</td>
<td>110.2</td>
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<tr>
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<td></td>
<td>122.0</td>
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<td>12b</td>
<td></td>
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<td>-</td>
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<td>56.0</td>
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<tr>
<td>9-OH</td>
<td></td>
<td>8.70, br s</td>
<td></td>
<td>-</td>
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</table>
Figure 141. HR-ESI mass spectrum of compound 13

Figure 142. UV spectrum of compound 13
Figure 143. IR spectrum of compound 13

Figure 144. $^1$H-NMR spectrum of compound 13 (400 MHz, acetone-$d_6$)
Figure 145. $^1$H-NMR spectrum of compound 13
(expansion between $\delta_H$ 6.7-9.0 ppm)

Figure 146. $^{13}$C-NMR spectrum of compound 13 (100 MHz, acetone-$d_6$)
Figure 147. $^{13}$C-NMR spectrum of compound 13
(expansion between $\delta_C$ 95-165 ppm)

Figure 148. $^1$H-$^{13}$C HSQC spectrum of compound 13
Figure 149. $^1$H-$^{13}$C HMBC spectrum of compound 13

Figure 150. $^1$H-$^{13}$C HMBC spectrum of compound 13
(expansion between $\delta_H$ 6.7-9.0 ppm, $\delta_C$ 80-165 ppm)
**Figure 151.** $^1$H-$^1$C HMBC spectrum of compound 13
(expansion between $\delta_H$ 10.2–11.0 ppm, $\delta_C$ 110-150 ppm)

**Figure 152.** $^1$H-$^1$H COSY spectrum of compound 13
Figure 153. $^1$H-$^1$H NOESY spectrum of compound 13
(expansion between $\delta_{^1H}$ 6.0-11.5 ppm)

Figure 154. $^1$H-$^1$H NOESY spectrum of compound 13
4.3 Inhibition of nitric oxide production in LPS-induced macrophages RAW 264.7 by isolated compounds

All compounds from *C. micrantha* stems and *M. siamensis* roots were tested for inhibition of nitric oxide production in LPS-induced macrophage RAW 264.7 cells. Among 5 compounds isolated from *C. micrantha*, (−)-pauciflorol E exhibited strong inhibitory activity with an IC$_{50}$ of 123.40 ± 4.51 μM, whereas methyl 6-methoxy-3-indolecarbonate inhibited NO production with an IC$_{50}$ of 198.00 ± 5.57 μM (Table 17). Interestingly, the stilbene dimer [(−)-pauciflorol E], having a keto carbonyl at position 8a, possessed NO inhibition activity, whereas the 8a-hydroxy substituted stilbene dimer ((+)-ampelopsin A) was inactive. The NO inhibitory activity of (−)-pauciflorol E, methyl 6-methoxy-3-indolecarbonate, and (−)-syringaresinol was first revealed in this study.

Table 17. Inhibitory concentrations of isolated compounds from *C. micrantha* stems on nitric oxide (NO) production and cell viability in LPS-induced RAW 264.7 cells

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC$_{50}$ of NO inhibition (μM)$^a$</th>
<th>Cytotoxicity (μM)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>methyl 6-methoxy-3-indolecarbonate</td>
<td>198.00 ± 5.57**</td>
<td>&gt;200</td>
</tr>
<tr>
<td>(compound 1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>vanillic acid (compound 2)</td>
<td>no activity at 50</td>
<td>no toxicity at 50</td>
</tr>
<tr>
<td>(−)-syringaresinol (compound 3)</td>
<td>284.80 ± 7.16&quot;</td>
<td>&gt;200</td>
</tr>
<tr>
<td>(+)-ampelopsin A (compound 4)</td>
<td>no activity at 50</td>
<td>no toxicity at 50</td>
</tr>
<tr>
<td>(−)-pauciflorol E (compound 5)</td>
<td>123.40 ± 4.51**</td>
<td>&gt;200</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>166.30 ± 6.24</td>
<td>&gt;200</td>
</tr>
</tbody>
</table>

** $p<0.001$ versus indomethacin (positive control)

$^a$ The IC$_{50}$ of NO inhibition was expressed as Mean ± SEM (standard error of the mean) from three independent experiments.

$^b$ The maximum concentration of test compounds was 200 μM.
As for *M. siamensis*, maeruabisindole B showed the strongest NO inhibition (IC$_{50}$ 31.1 ± 1.0 μM) among the isolates from *M. siamensis* roots, followed by maeruabisindole B (IC$_{50}$ 56.7 ± 2.2 μM), maeroxide C (IC$_{50}$ 92.2 ± 5.1 μM), (+)-maeruanitrile A (IC$_{50}$ 186.4 ± 13.0 μM) and maeruanitrile B (IC$_{50}$ 186.8 ± 13.3 μM), compared to indomethacin (IC$_{50}$ 150.0 ± 16.0 μM, a positive control) (Table 18). Most notably bisindole alkaloids exhibited anti-inflammatory activity; for example, isatindigosides F and G from *Isatis tinctoria* roots (Brassicaceae) showed NO inhibitory activity at IC$_{50}$ of 70.3 ± 6.9, and 67.3 ± 5.5 μM, respectively (Dongdong Zhang *et al.*, 2020). In addition, indole-3-acetonitrile compounds isolated from *Isatis indigotica* roots such as indole-3-acetonitrile, arvelexin, 1-methoxy-indole-3-acetonitrile also demonstrated the NO inhibitory activity (Yang *et al.*, 2014).
Table 18. Inhibition concentrations of isolated compounds from *M. siamensis* roots on nitric oxide (NO) production and cell viability in LPS-induced RAW 264.7 cells

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC$_{50}$ of NO inhibition (µM)$^a$</th>
<th>Cytotoxicity (µM)$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)-maeruanitrile A (compound 6)</td>
<td>186.4 ± 13.0</td>
<td>&gt;200</td>
</tr>
<tr>
<td>maeruanitrile B (compound 7)</td>
<td>186.8 ± 13.3</td>
<td>&gt;200</td>
</tr>
<tr>
<td>maeroxime A (compound 8)</td>
<td>n.d.$^b$</td>
<td>toxicity at 100</td>
</tr>
<tr>
<td>maeroxime B (compound 9)</td>
<td>&gt;200 (231.2 ± 11.6 *** )</td>
<td>&gt;200</td>
</tr>
<tr>
<td>maeroxime C (compound 10)</td>
<td>92.2 ± 5.1**</td>
<td>&gt;200</td>
</tr>
<tr>
<td>maeruabisindole A (compound 11)</td>
<td>n.d.$^b$</td>
<td>toxicity at 100</td>
</tr>
<tr>
<td>maeruabisindole B (compound 12)</td>
<td>31.1 ± 1.04****</td>
<td>toxicity at 100</td>
</tr>
<tr>
<td>maeruabisindole C (compound 13)</td>
<td>56.7 ± 2.2****</td>
<td>&gt;200</td>
</tr>
<tr>
<td>indomethacin</td>
<td>150.0 ± 16.0</td>
<td>&gt;200</td>
</tr>
</tbody>
</table>

$^a$The IC$_{50}$ of NO inhibition was expressed as Mean ± SEM from three independent experiments.

$^b$n.d. refers to ‘not determined’. The compound could not be determined for IC$_{50}$ value due to its cytotoxicity.

$^c$The maximum concentration of test compounds was 200 µM. Cytotoxic was indicated by the concentration given that cell viability was lower than 80%. The tested concentration that exhibited cell viability below 80% was noted as “toxicity at that concentration.”

**p < 0.005  ***p < 0.001 and ****p < 0.0001 versus indomethacin (positive control).
CHAPTER V
CONCLUSION

Phytochemical investigation of *Capparis micracantha* stems and *Maerua siamensis* roots led to the isolation of five known compounds [methyl 6-methoxy-3-indolecarbonate, vanillic acid, (−)-syringaresinol, (+)-ampelopsin A, and (−)-pauciflorol E] from *C. micracantha* and eight new compounds named (+)-maeruanitrile A, maeruanitrile B, maeroximes A − C, and maeruabisindoles A − C from *M. siamensis*. For nitric oxide inhibition assay in LPS-induced macrophages RAW 264.7, (−)-pauciflorol E, methyl 6-methoxy-3-indolecarbonate, (−)-syringaresinol from *C. micracantha* exhibited the activity at IC<sub>50</sub> of 123.40 ± 4.51, 198.00 ± 5.57 and 284.80 ± 7.16 µM, respectively. In addition, maeruabisindole B, maeruabisindole C, maeroxime C, maeruanitrile A, and maeruanitrile B displayed nitric oxide inhibition at IC<sub>50</sub> of 31.1 ± 1.04, 56.7 ± 2.2, 92.2 ± 5.1, 186.4 ± 13.0, 186.8 ± 13.3, respectively, while an IC<sub>50</sub> of indomethacin (a drug for anti-inflammation) is in the range of 150.0 – 166.3 µM.

These findings reveal the anti-inflammatory compounds in *C. micracantha* stems and *M. siamensis* roots, which are herbal drugs used for the treatment of inflammation according to Thai traditional medicines and support the use of this herbal drug. Moreover, the promising nitric oxide inhibitory compounds (maeruabisindoles B and C) could be developed for the potent anti-inflammatory agents in the future.

Furthermore, this study expands knowledge in chemotaxonomy regarding plants in Capparaceae; for example, stilbene oligomers and lignans in *Capparis* species and glucosinolate-derived indole alkaloids and bisindole alkaloids in *Maerua* plants. Lastly, indole alkaloids which is similarly found in plants in the family Brassicaceae, it could be used as chemotaxonomic marker between Capparaceae and Brassicaceae, which are closely related family.
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