CHEMOTAXONOMICAL EVALUATION OF THE RED ALGA LAURENCIA NANGII MASUDA (RHODOMELACEAE, CERAMIALES) IN THE COASTAL WATERS OF SABAH

TAN KAI LEE

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DECLARATION

I hereby declare that this thesis contains my original research work. Sources of findings reviewed herein have been duly acknowledged.

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TAN KAI LEE
PS05-005-008
CERTIFICATION

NAME : TAN KAI LEE

TITLE : CHEMOTAXONOMICAL EVALUATION OF THE RED ALGA LAURENCEA NANGII MASUDA (RHODOMELACEAE, CERAMIALES) IN THE COASTAL WATERS OF SABAH

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1. ASSOC. PROF. DR. CHARLES S. VAIRAPPAN
   SUPERVISOR
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ABSTRACT

CHEMOTAXONOMICAL EVALUATION OF THE RED ALGA
Laurencia nangii Masuda (RHODOMELACEAE, CERAMIALES)
IN THE COASTAL WATERS OF SABAH

Species discrimination in the red alga genus Laurencia is difficult due to a high degree of morphological variation within individual species. Chemical studies on a worldwide basis revealed that most species of Laurencia can be characterized by a specific set of halogenated secondary metabolites. Some species, however, produce unrelated sets of metabolites depending on geographical distribution. The morphologically similar but chemical distinct populations can be referred to as sibling species or chemical races. This study was carried out to identify the halogenated secondary metabolites that can be utilized as taxonomic markers for Laurencia nangii Masuda. Samples of L. nangii from three localities: Pulau Sulug, Kota Kinabalu; Pantai Bak-Bak, Kudat; and Dogoton, Pulau Banggi, Kudat were investigated. Voucher herbarium specimens of each population (BORH 37572–37577, 37587–37588) were deposited in the Borneensis Collection in the Institute for Tropical Biology and Conservation. Plants are purplish-green in colour and attain height of 3 – 6.5 cm, with several upright axes arise from discoid holdfast and stolon-like branches. Microscopy study showed that superficial cortical cells contain two to three corps en cerise, while trichoblast cells contain one or two corps en cerise. Tetrasporangia are formed on all four orders of branches and measure 100 – 120 μm in diameter. Ovoid cystocarps are formed on first- to fourth-order branches, measure 550 – 800 μm in height by 500 – 750 μm in diameter. Male trichoblasts are formed in the cup-shaped apical depression of first- to fourth-order branches. Samples were extracted and the crude extracts from these three populations showed different chemical profiles on TLC and RP-HPLC. Crude extracts were fractionated by column chromatography and isolation of secondary metabolites was carried out via RP-HPLC. The structures of the isolated compounds were deduced from 1H-NMR, 13C-NMR, and 2D NMR (COSY, HSQC, HMBC and NOESY) spectra. Three C15-acetogenins (C1 – C3) were isolated from Pulau Sulug's sample. C1 present in 2.4% of crude extract, C2, 2.0% and C3, 1.0%. Five Cw acetogenins (C4 – C8) were isolated from Pantai Bak-Bak samples. C4 present in 1.8%, C5, 1.3%, C6, 2.6%, C7, 1.1% and C8, 1.9%. And, one C15-acetogenin, C9 (15.0%) was isolated from Dogoton's sample. Thus it is conclusive that L. nangii is characterized by C15-acetogenins and they could serve as chemotaxonomic markers for the species. Since these compounds are derived from similar metabolic pathway, sibling species or chemical races may not be present in this species.
ABSTRAK

Diskriminasi spesis alga merah genus Laurencia adalah sukar disebabkan oleh perbezaan morfologi yang wujud dalam sesuatu spesis. Kajian atas genus ini di pelosok dunia menunjukkan bahawa kebanyakan spesis dapat dicirikan dengan suatu set metabolit sekunder berhalogen yang tersendiri bagi sesuatu spesis tersebut. Namun, terdapat juga spesis yang menghasilkan set metabolit sekunder yang berlainan bergantung pada taburan geografi. Spesis sebagian yang mempunyai ciri morfologi yang sama tetapi kandungan sebatian kimia yang berlainan dikatakan mempunyai ras kimia (chemical race). Kajian ini dilakukan untuk mengenalpasti metabolit sekunder berhalogen yang boleh dijadikan sebagai petanda taksonomi bagi Laurencia nangii Masuda. Persampelan dilakukan di tiga lokasi di persisiran pantai Sabah, iaitu: Pulau Sulug, Kota Kinabalu; Pantai Bak-Bak, Kudat; dan Dogoton, Pulau Banggi, Kudat. Specimen herbarium untuk setiap populasi (BORH 37572–37577, 37587–37588) didepositkan dalam Koleksi Borneensis di Institut Biologi Tropika dan Pemuliharaan. Talus berwarna hijau-keunguan dan mencapai ketinggian 3.0 – 6.5 cm; beberapa cabang tegak muncul dari plelekap berbentuk cakera dan dari cabang stolon. Kajian mikroskopi menunjukkan sel kortikal permukaan mengandungi dua hingga tiga ‘corps en cerise’; manakala sel trikoblas mengandungi satu atau dua ‘corps en cerise’. Tetrasporangium yang berukuran 100–120 μm membentuk pada anak cabang. Sistokarpa berbentuk bulu dengan ketinggian 550 – 800 μm dan diameter 500 – 750 μm. Trikoblas jantan membentuk dalam liang pada hujung anak cabang. Sampel dari ketiga-tiga populasi diekstrak dan profil kimia (TLC dan HPLC) ekstrak karas menunjukkan corak yang berlainan. Pembahagian ekstrak kasar dilakukan melalui kromatografi kolum dan pemencilan metabolit sekunder dilakukan melalui RP–HPLC. Struktur kimia ditentukan melalui kaedah spektroskopi 1H,13C-RMN, dan RMN 2D (COSY, HSQC, HMBC and NOESY). Tiga sebatian C15-acetogenin (C1–C3) dipencikan dari sampel dari Pulau Sulug: C1, 2.4%, C2, 2.0%, C3, 1.0%; lima sebatian C15-acetogenin (C4–C8) dipencikan dari sampel dari Pantai Bak-Bak: C4, 1.8%, C5, 1.3%, C6, 2.6%, C7, 1.1%, C8, 1.9%; dan satu sebatian C15-acetogenin, C9 (15%) dipencikan dari sampel dari Dogoton. Daripada kajian ini, adalah didapati bahawa L. nangii dicirikan dengan sebatian C15-acetogenin yang berasal dari laluan metabolik yang sama, maka ras kimia tidak wujud dalam spesis ini.
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CC          Column chromatography
CDCl₃       Deuterated chloroform
dH₂O        Distilled water
EtOAc       Ethyl acetate
 Et₂O       Diethyl ether
FTIR        Fourier transform infrared
HSQC        Heteronuclear single quantum coherence
HMBC        Heteronuclear multiple bond correlation
HREIMS      High resolution electron ionization mass spectrometry
LREIMS      Low resolution electron ionization mass spectrometry
KBr         Potassium bromide
MeOH        Methanol
MeCN        Acetonitrile
Mp          Melting point
Na₂SO₄      Anhydrous sodium sulphate
NOESY       Nuclear Overhauser effect spectroscopy
RP-HPLC     Reverse phase High performance liquid chromatography
Rᵣ          Mobility relative to front
Tᵣ          Retention time
Si          Silica
TLC         Thin layer chromatography
TMS         Tetramethylsilane
UV-Vis      Ultra violet – visible
¹H-¹H COSY  Proton-proton correlation spectroscopy
¹H-NMR      Proton nuclear magnetic resonance
¹³C-NMR     Carbon-13 nuclear magnetic resonance
2D          Two dimensions
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CHAPTER 1

INTRODUCTION

1.1 Red Algae Genus *Laurencia*

Marine red algae genus *Laurencia* was first described by Lamouroux in the year 1813. It is a relatively large genus with approximately 135 species, which spans a wide geographical range, having been documented in tropical, sub-tropical and temperate waters (Masuda *et al.*, 1996). It is classified under the phylum Rhodophyta, class Rhodophyceae, subclass Florideophyceae, order Ceramiales, family Rhodomelaceae.


*Corps en cerise* are intracellular refractive globular inclusions, which distribute exclusively in superficial cortical cells and trichoblast cells, where halogenated secondary metabolites are synthesized and/or stored (Young *et al.*, 1980). The presence or absence and their number have a valuable taxonomic meaning at specific level within the genus *Laurencia* (Furnari *et al.*, 2001).

### 1.2 Chemotaxonomy Study of *Laurencia*

The genus *Laurencia* has attracted intense interest among organic chemists due to its ability to produce diverse types of halogenated secondary metabolites. The first chemical investigation on the red algae *Laurencia* was done in 1953 when Obata and Fukushi studied the chemistry of *Laurencia nipponica* Yamada (as *Laurencia glandulifera* Kütznig) from Oshoro Bay, Hokkaido, Japan and reported the odoriferous components of the essential oil to be sesquiterpenoids. In 1963, Irie and coworkers started their continuous studies on the chemical constituents of *Laurencia* that led to the isolation of Laurencin, the first brominated acetogenin, from the same alga collected at the same location in 1965. They isolated laurinterol and debromolaurinterol from *L. okamurae* Yamada (as *L. intermedia* Yamada) the following year. Since then, various groups around the world have carried out research on this aspect of the algae. To date, more than 500 diverse and unique halogenated secondary metabolites, in particular, sesquiterpenoids, diterpenoids, triterpenoids and C_{15} acetogenins, have been discovered from more than 60 *Laurencia* species worldwide (Suzuki and Vairappan, 2005).

Various studies (Erickson, 1983; Suzuki and Vairappan 2005) led to the observation that, while there is a certain degree of overlap, most species in this genus can be characterized by at least one specific compound not found in the others or a particular set of compounds, render them as useful taxonomic characters for species discrimination. In the year 1975, Fenical and Norris
differentiated three different entities passing under the name of Laurencia pacifica Kylin from the Gulf of California based on comparative thin layer chromatography of lipid components. Progressively, in the year 1980, Howard et al. investigated a few species of Laurencia under culture and found that the production of secondary metabolites were unaffected by varying photoperiod and temperature conditions and the chemical compounds produced were both quantitatively and qualitatively identical to those of the natural populations.

It is noted too that some species produce unrelated sets of metabolites depending on geographical distribution, i.e. each chemical types is characterized by a specific end product of halogenated secondary metabolism. In the case of morphologically similar but chemical distinct populations, these can be referred to as sibling species or chemical races. The difference between these two is that, reproductive isolation is presence among differentiated populations for the formal, as exemplified by L. pacifica Kylin (Fenical and Norris, 1975); and the opposite for the latter. L. nipponica Yamada is the classical example of a species that has several chemical races, the first report in marine algae (Masuda et al., 1997a). By virtue of their stability of specificity, halogenated secondary metabolites proved to be useful taxonomic feature at species level when the metabolites are comprehensively studied throughout the algal geographical range (Masuda et al., 1997a; Suzuki and Vairappan, 2005).

1.3 Laurencia species in Malaysian Waters

Documentations on the genus Laurencia in Malaysian waters are relatively limited (Ismail, 1995; Masuda et al., 2001; Yamagishi et al., 2003). Even fewer reports were published on its chemical compositions: L. snackeyi (Masuda et al., 1997b), L. similis (Masuda et al., 1999; Vairappan et al., 2004), L. nangii (Masuda et al., 2002a), L. majuscula (Vairappan et al., 2001a, 2003), L. pannosa (Suzuki et al., 2001), and L. pygmeae (Vairappan et al., 2001a; Yamagishi et al., 2003).

Laurencia nangii Masuda (Figure 1.1) is a relatively new species originally described from Vietnam (Masuda, 1997) and was reported from Malaysian water as recent as the year 2002 (Masuda et al., 2002a). It is readily recognizable by its
fleshy and soft, green colour thalli. It is characterized by several upright terete, percurrent axes (2 – 6 cm in length) arise from a common discoid holdfast and from stolon-like branches, and produce first-order branches, which are arranged in an irregular spiral and bear progressively shorter branches of up to four order. Our survey (unpublished data) revealed that this species is widely distributed along the coastal waters of Sabah, commonly found growing epiphytically on various algae or on dead coral in the lower intertidal to upper subtidal zone.

Figure 1.1: Laurencia nangii Masuda

Masuda et al. (2002) reported the isolation of three C₁₅ acetogenins: cis-pinnatifidenyne (1), obtusenyne (2) and (3Z)-laurenyn (3) from a sample collected from Pulau Tiga, Kuala Penyu; while a sample from Pulau Bai, Sandakan, contained (1) and (2) and diterpenoid, aplysiadiol (4) instead of (3). Figure 1.2 shows the chemical structures of (1) – (4). The authors suggested the presence of two chemical races in this species in its Malaysian populations: 1) a race characterized by the production of only acetogenins and distributed on the west coast of Sabah, and 2) another race characterized by the production of acetogenins along with aplysiadiol (diterpenoid) on the east coast of Sabah.
To date, this is the only report published on the chemical composition of *L. nangii*. Hence, present research is undertaken to identify chemotaxonomical markers for *Laurencia nangii* Masuda as part of the effort to document the distribution and the chemical composition of red algae genus *Laurencia* in Malaysian waters.

### 1.4 Objectives

The objectives of this study are:

1. To describe morphological features of *L. nangii*.
2. To quantify crude extract contents and chemical profiles of *L. nangii* collected from three localities in the coastal waters of Sabah.
3. To isolate and characterize halogenated secondary metabolites from these three populations.
2.1 Distribution and Chemical Diversity of Laurencia Species

Red algae of the genus Laurencia produce a wide variety of structurally unusual secondary metabolites. These metabolites can be conveniently classified into four structural classes: sesquiterpenoids, diterpenoids, triterpenoids and C$_{15}$ acetogenins. Due to the vast number of species and the compounds they produce, selected species are included in this review to demonstrate how these compounds have aided in species discrimination in this taxonomically troublesome genus. Information regarding the geographical distribution of the species mentioned in section 2.1.1 – 2.1.11 below was obtained from Guiry and Guiry, 2007.

2.1.1 Laurencia brongniartii Agardh

Laurencia brongniartii has been reported from various localities of tropical to subtropical regions in the world: Ireland, Canary Islands, Cuba, Ghana, Madagascar, South Africa, the Philippines, Sri Lanka, Japan, Taiwan, Indonesia, Australia and New Zealand.

This species is characterized by polyhalogenated indoles. Sample collected in the Caribbean Sea was found to produce 1-methyl-2,3,5,6-tetrabromoindole (5), 2,3,5,6-tetrabromoindole (6), 1-methyl-2,3,5-tribromoindole (7), 1-methyl-2,3,6-tribromoindole (8). One the other hand, a Taiwanese population and a southern Japanese population were reported to contain four methylthiobromoindoles in common: (9), (10), (11) and (12). The Japanese population further contains two methylsulfinylbromoindoles: Itomanindole A (13) and itomanindole B (14), two simple bromoindoles: (15) and (16) and methylthiobromoindoles (17) and (18) (Masuda et al., 1999; Suzuki and Vairappan, 2005). Figure 2.1 shows the chemical structures of (5) – (18).
2.1.2 Laurencia composita Yamada

Laurencia composita has been reported from Caribbean Islands, the Philippines and several localities along the Pacific coasts of Japan. This species is characterized by oxygenated chamigrane-type sesquiterpenoids. Masuda et al. (1996) reported pacifenol (15), prepacifenol epoxide (16) and johnstonol (17) from this species collected at Matsuura, Nagasaki Prefecture (Pref.), Awaji, Hyogo Pref. and Tateyama, Chiba Pref. Population collected from Tanegashima Island, Kagoshima Pref. also contains these three compounds along with another two chamigrenes: 2,10-dibromo-3-chloro-α-chamigrene (18) and 2,10-dibromo-3-chloro-9-hydroxy-α-chamigrene (19) (Masuda et al., 2002b). Earlier on, Suzuki and Kurosawa (1985) confused this species as L. okamurae when they reported that the population from Iwaizaki, Mie Pref. contains two sets of sesquiterpenoids, chamigranes (pacifenol,
REFERENCES:


