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Rozi Mohamed Editor

Agarwood

Science Behind the Fragrance



Tropical Forestry

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Agarwood

Science Behind the Fragrance



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Preface

Agarwood is the contemporary name given to this fragrant natural product from trees of the Thymelaeaceae family, the most widespread being *Aquilaria*. This ancient product has provided a great sense of appreciation to humankind in many ways, from incenses used in rituals by different societies around the world to traditional medicines and fragrances. In modern days, agarwood still performs its long acclaimed services but with a renewed interest as it gains popularity in cosmetics and medicine manufacturing. Being heavily sourced from the wild, agarwood resources are quickly depleting due to habitat demolition and destructive harvesting techniques. In recent years, *Aquilaria* plantations have received increasing attention as a renewable source of agarwood for various downstream industries.

Agarwood fragrance is distinctive that it is often associated with myths and spiritual experiences. This book is dedicated to understanding the facts about agarwood formation, hence the title *Science Behind the Fragrance*. Despite its centuries-old existence, the biology of agarwood resources has been very much eluded. Domestication efforts of the tree provide promising new developments in agarwood induction and detection technologies. Scientists have identified critical components that affect agarwood productivity resulting from the tree host reaction to biotic and abiotic stresses. Advances in biotechnology and genomics of *Aquilaria* species have shed light on the process of its cell machinery in synthesizing crucial compounds in agarwood, many of which are responsible for the unique fragrance. New tree resources have been explored, and new pharmaceutical properties have emerged. All these are covered in the chapters, providing researchers and the public with a sourcebook of current knowledge on agarwood.

Most of the chapter authors participated in the 1st International Scientific Symposium on Agarwood (ISSA), *Agarwood in the New Era*, convened in 2013 at the Universiti Putra Malaysia (UPM), Serdang campus. Organized by the Faculty of Forestry, UPM, with collaboration from the Forest Research Institute Malaysia (FRIM), the Asia Pacific Association of Forestry Research Institutions (APAFRI), the Malacca State Forestry Department, and the Malaysian Timber Industry Board (MTIB), together with compassionate sponsors, ISSA2013 provided a platform for the genesis of this book.

I especially thank all chapter authors for their valuable contributions. This book was designed to nurture information exchange and to inspire agarwood scientists. I hope this publication will serve as a basis for future research with agarwood tree species and other aromatic tropical tree genera.

UPM Serdang, Malaysia January 2016 Rozi Mohamed

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Chapter 1 The Origin and Domestication of *Aquilaria*, an Important Agarwood-Producing Genus

Shiou Yih Lee and Rozi Mohamed

Abstract The Aquilaria (Thymelaeaceae) tree is a well-known important agarwood-producing genus, which is endemic to the Indomalesia region. The genus is currently protected under CITES regulation and the IUCN Red List due to its heavy declination in the natural population in various sourcing countries. Derived from its precious non-wood fragrant products, the genus was given different names throughout the history until it was finalized in 1783. To date, there are 21 recognized Aquilaria species recorded, of which 13 are reportedly fragrant resin producers, and the status of the remaining eight Aquilaria species is yet to be investigated. Aquilaria is heavily exploited in the wild due to the destructive agarwood harvesting technique that requires hacking of the wood parts to induce agarwood production. Various conservation efforts have been carried out to avoid further destruction toward its gene pool. This includes introducing the species for cultivation and planting the trees in large plantations or home gardens, which further provide a sustainable agarwood production in the industry and indirectly contribute to the local economy. At present, an accurate classification of Aquilaria species is yet to be achieved; misidentification happens frequently, either genuinely because of lack of information and training or intentionally for business gains. In conclusion, a proper taxonomy and classification system are essential for conserving Aquilaria species genetic diversity and for identifying species origin of agarwood products aimed at international trade control.

1.1 Introduction

The *Aquilaria* genus is well known for its fragrant non-wood product, the agarwood. Highly demanded in several countries, agarwood is further processed into perfumes, incenses, and ornamental displays and used as a raw material in traditional and modern medicines. Historically, human's encounter with agarwood was first

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recorded in ancient literatures and religious scriptures. The Sanskrit language poet, Kâlidâsa (c. 353–c. 420), once wrote: *Beautiful ladies, preparing themselves for the feast of pleasures, cleanse themselves with the yellow powder of sandal, clear and pure, freshen their breast with pleasant aromas, and suspend their dark hair in the smoke of burning aloes.* The word "aloes" has the same meaning as agarwood. It was also found occurring in the biblical text (Duke 2007). In ancient days, the Egyptians used agarwood to embalm honored dead bodies, and in several Asian countries, agarwood products were introduced along with Buddhism from India. During those days, the most familiar *Aquilaria malaccensis*) from India and nearby countries and *Ophispermum sinense* (synonym to *Aquilaria sinensis*) from China (Don 1832). The former was widely applied in fragrance production and religious practices while the latter in Chinese medicines.

From a total of 21 accepted species names for *Aquilaria* at present (The Plant List 2013), about thirteen are reported as fragrant resin producer: *A. baillonii*, *A. beccariana*, *A. crassna*, *A. filaria*, *A. hirta*, *A. khasiana*, *A. malaccensis*, *A. microcarpa*, *A. rostrata*, *A. rugosa*, *A. sinensis*, *A. subintegra* and *A. yunnanensis* (Hou 1960; Ng et al. 1997; Compton and Zich 2002; Kiet et al. 2005; Yang Y 2015, personal communication). As for the remaining eight *Aquilaria* species, their competencies at producing agarwood need to be further investigated.

1.2 A Brief History of the Genus

The genus name, *Aquilaria*, was originally derived from its non-wood fragrant product, presently known as agarwood. Agarwood has many names that have been passed down over many generations. When the product is introduced into various societies, each mimicked the pronunciation of the original name using her own language; thus, more names were created. From recorded history, the earliest name given was *ahalim* in Hebrew and *ahila* from the Scripture of the East (Ridley 1901), followed by *agalukhi* in Arabic. It was also described as *agallochee*, a Greek synonym to a Hindi word for incense wood *aod-i-kimaree* from India and Arabia. In the Malayan region, it has been known as *agila*, which possibly descended from the Sanskrit *agara* (in Hindi *aggur*). In more recent days, the Portuguese gave several names: *pao-d'agila*, *pao-d'aguila*, *pao-d'aquila*, *bois d'aigle*, *eagle-wood*, and *agel-hout*. The genus established its final name, *Aquilaria*, in 1783, given by the botanist Jean-Baptiste Lamarck after replacing its synonym, *Agallochum* of Dioscorides.

The first scientific record of agarwood usage was likely that of Avicenna, an Arab physician (980–1037), who described several types of *agallochum* (Society for the Diffusion of Useful Knowledge 1838). Among the different types of *agallochum*, he recorded two names, *xylaloes* and *agalugen*. *Xylaloes* is a Greek form of an Arabic word *alud*, literary described as "the wood." Further, it was modified into aloe wood/aloes-wood and also Lignum aloes. As for *agalugen*, it was also called *aghaloojee*, which was then defined as *agallochee* or *agallochum*. The word

agallochum was simplified to *agalloch*, referring to the fragrant wood produced from *A. agallocha* in India.

The first formal account to the tree itself was by Garcia de Orta (1501-1568), a Portuguese Renaissance physician and naturalist, who practiced in Goa, India, and was a pioneer of tropical medicine (Ridley 1901). He visited Malacca in Peninsular Malaysia roughly in 1534 and named the fragrant wood garo. He recorded that the wood was brought by the Chinese from Malacca and Sumatra; thus, he referred it as Garo de Malacca. Garcia successfully collected the twigs and leaves from trees growing in Malacca but failed to get the fruits or flowers as there was difficulty to access the forest. Georg Eberhard Rumphius (1627-1702), a German-born botanist who was studying on the specimen brought back from Malacca, distinguished two types of agallochum: the calambac (Agallochum primarium) and the garo (Agallochum secundarium) (Society for the Diffusion of Useful Knowledge 1838). The calambac or calembouc (French) had other names such as ky nam (Vietnam) and kyara (Japanese) (Li 1998). The first collection of calambac was native to Eastern Cochin China and Siam, collected by Loureiro from the tree called Aloexylum agallochum Lour., while the second collection was native to Cochin China and Laos, also collected by Loureiro from the tree called Ophispermum sinense Lour. (known as A. sinensis at present) (Ridley 1901). William Roxburgh (1751–1815), a Scottish surgeon and botanist, who is known as the Father of Indian Botany, described that the real calambac comes from A. agallocha Roxb., which was exported to China from the eastern frontier instead of Cochin China. Calambac from both origins had equal demands and were growing at similar latitudes, yet no one could conclude that they derived from the same species because botanical description was incomplete at that time (Society for the Diffusion of Useful Knowledge 1838).

Garo was later known as garos, which was recorded as an article of export from Malacca and the kingdom of Siam (Thailand at present). It was given the name garu (in Malay, later updated to gaharu), derived from the Sanskrit aquaru but only referring to the fragrant wood. The garu tree is given a different name and is known as karas, tuikaras, tengkaras, engkaras, and kakaras by the Malays (Ridley 1901). Pierre Sonnerat (1748–1814), a French naturalist and explorer, successfully obtained the specimens of the tree during his second voyage to India, based on the figures and description done by Jean-Baptiste Lamarck (1744-1829) for the bois d'aloes, Agallochum officinarum. Lamarck concluded that the collected specimen greatly resembles the A. secundarium from Rumphius; thus, it was renamed as A. malaccensis (Royle 1839). Upon confirmation, Francis Hamilton (1762–1829, also known as Francis Buchanan) concluded that A. malaccensis and A. agallocha are both of the same tree in nature but prefers the name A. officinarum as the official name for the plant (Hamilton 1836). Today, the name A. malaccensis retains as a type specimen for the genus Aquilaria in taxonomy identification. Since the official genus Aquilaria was agreed upon in 1783, it replaced several synonym genera including Agallochum Lam. (1783), Aloexylum Lour. (1790), Aquilariella Tiegh. (1893), Decaisnella Kuntze (1891), Gyrinopsis Decne. (1843), and Ophispermum Lour. (1790) (Tropicos 2016).

1.3 Generic Status and Relationships

Aquilaria is a member of the Thymelaeaceae (Malvales) family and belongs to the subfamily Thymelaeoideae (previously Aquilariodeae). There was a minor controversy in the classification of the subfamilies in Thymelaeaceae. Until today, a stable circumscription in the taxon of Thymelaeaceae is yet to be achieved. Conventional classification of the Thymelaeaceae is always related to the identification through morphological and reproductive characteristics of the plant itself. Earlier in 1836, before an international taxonomy system was established, Aquilaria was once under the order Aquilariaceae, Alliance Daphnales. However in 1880, the Bentham and Hooker system removed the genus on reasons that there was no recollection exercise since previous identification (Watt 2014). Years later, it was re-added, together with Gyrinopsis and Gyrinops into the family Thymelaeaceae, under subfamily Aquilarioideae (including Phalerioideae, Thymelaeoideae, and Drapetoideae) and tribe Aquilarieae (Gilg 1894). Thymelaeaceae was under the order of Thymelaeales. Later in 1967, Hutchinson proposed to include Aquilariaceae under Thymelaeales. However in 1968, Cronquist proposed to embed Thymelaeaceae under the order Myrtales, then further suggested by Thorne to place under Euphorbiales, instead of Myrtales. The debate between Myrtales and Euphorbiales was ongoing from 1968 to 1993, with a small different opinion whereby Cronquist's proposal was accepted from 1988 to 1992, in which Thymelaeaceae was placed as the sole family under the order Thymelaeales (Cronquist 1988). In 1993, Heywood placed Thymelaeaceae under the order Myrtales after removing it from the previous order Euphorbiales, which was proposed by Thorne in 1992. In 1998, the Angiosperm Phylogeny Group included the family Thymelaeaceae in the Malvales order, disregarding indication toward families adjacent to it (Angiosperm Phylogeny Group 1998). Chronology of the events is shown in Table 1.1.

Subfamilies within the Thymelaeaceae were not established as well throughout the centuries. As such in 1894, Gilg proposed four subfamilies under Thymelaeaceae: Aquilarioideae, Phalerioideae, Thymelaeoideae, and Drapetoideae. However in 1921, he reviewed the list and added three new subfamilies: Microsemmatoideae, Octolepidoideae, and Synandrodaphnoideae. Domke (1934) scaled down the list by retaining two names and adding two new ones: Aquilarioideae, Gilgiodaphnoideae, Gonystyloideae, and Thymelaeoideae. The latest update in the subfamilies within the Thymelaeaceae was by Herber in 2002 and 2003, concluding only two big subfamilies: Octolepidoideae and Thymelaeoideae, with Aquilarieae placed under Thymelaeoideae. At present, *Aquilaria* and its closely related genus *Gyrinops* are under the order Malvales, family Thymelaeaceae, subfamily Thymelaeoideae, and tribe Aquilarieae.

The four-subfamily classification, proposed by Gilg (1894), was mostly supported by molecular phylogeny as revealed from the sequences of the chloroplast DNA, the *rbcL* gene and the *trnL-trn*F intergenic spacer region, of forty-one samples under the Thymelaeaceae (Van der Bank et al. 2002). Unfortunately, the phylogenetic relationship was not supported at level of tribe. The study was further investigated by

	Year			
Taxonomist	proposed		Changes made	
Lidney	1836	Alliance Order Genera	Daphnales Aquilariaceae; Elaeagnaceae; Hernandiaceae Thymelaeaceae; Aquilaria; Gyrinops; Ophiospermum	
Bentham and Hooker	1880	Genera	Aquilaria was removed	
Gilg	1894	Subfamily Tribe Genera	Aquilarioideae;Thymelaeoideae;DrapetoideaeAquilarieaeAquilaria;Gyrinopsis;Gyrinopsis;	
Gilg	1921	Subfamily	Aquilarioideae; Phalerioideae; Thymelaeoideae; Drapetoideae; Microsemmatoideae; Octolepidoideae; Synandrodaphnoideae	
Domke	1934	Subfamily General Tribe Genera	Aquilarioideae; Gilgiodaphnoideae; Gonystyloideae; Thymelaeoideae Aquilariidae Aquilaria; Gyrinops	
Hutchinson	1959	Order	Thymelaeales	
Hutchinson	1967	Order Family Genera	Thymelaeales Aquilariaceae; Thymelaeaceae Aquilaria; Deltaria; Gyrinops; Lethedon; Octolepis; Solmsia	
Cronquist	1968	Order	Myrtales	
Thorne	1968	Order	Euphorbiales	
Cronquist	1988	Order	Thymelaeales	
Thorne	1992	Order	Euphorbiales	
Heywood	1993	Order	Myrtales	
The Angiosperm Phylogeny Group	1998	Order	Malvales	
Herber	2002	Subfamily Tribe Genera	Octolepidoideae; Thymelaeoideae Aquilarieae ; Daphneae; Synandrodaphneae Aquilaria ; Gyrinops	

Table 1.1 Chronology of events following changes in taxonomy affinities of the genus Aquilaria(Thymelaeaceae)

Rautenbach (2008) who performed molecular phylogenetic analysis using 143 specimens from the Thymelaeaceae members, which was three times greater in sample size compared to the previous study. The two regions from the chloroplast DNA were analyzed in addition to the nuclear ribosomal DNA internal transcribed spacer (ITS). The results were in support of the classification proposed by Herber in 2002. Considering that molecular phylogeny approach can provide remarkable results that complement conventional taxonomic classification in Thymelaeaceae, it has been applied for identifying agarwood-producing species from the genus *Aquilaria*. Ito and Honda (2005) sequenced the ITS1 and *psbC-trn*S regions in their study, while Eurlings and Gravendeel (2005) sequenced the *trnL-trn*F intergenic spacer region. Both studies used authentic samples from herbarium specimens and concluded that molecular-based approach is possible for identifying *Aquilaria* species. Eurlings and Gravendeel (2005) provided a wider scope when they included *Gyrinops* specimens and concluded that *Aquilaria* and *Gyrinops* are paraphyletic, indicating that the two genera had shared the last common ancestor. Although molecular-based study seems promising in assisting identification at genus and species levels, further studies are needed for conservation and trade control purposes.

1.4 Distribution of the Species

Aquilaria is widely distributed in the Indomalesia region. The most dominant species, which has its population over several countries, is *A. malaccensis*. The accepted species according to The Plant List, their distributions based on previous records, and their conservation status as classified by IUCN, are compiled in Table 1.2.

To help illustrate the distribution, an imaginary horizontal line parallel to the equator is drawn going from across the Sumatra Island to Borneo Island, and a vertical line is drawn from the east of Taiwan going through the west of the Philippines, separating Borneo from Sulawesi and west of Sumba Island (Fig. 1.1). For the benefit of this discussion, these crossing lines divide the Indomalesia region into four sections and reflect the distribution of the related species in a congruent manner. Starting with the northwest end, this first region is widely populated by A. crassna, A. malaccensis, and A. sinensis. The distribution of A. crassna has been reported in Cambodia, south of Laos, north of Thailand, and Cochin China of Vietnam; A. malaccensis in Bangladesh, Bhutan, Assam of northeast India, Sumatra and Kalimantan of Indonesia, Iran, Malaysia, Myanmar, south of the Philippines, Singapore, and south of Thailand; and A. sinensis meanwhile endemic to China, confined mainly to the south, Hainan Island, Hong Kong, and Taiwan. Besides that, records have shown that A. baillonii is endemic to Cambodia; A. banaensis to Bana of Vietnam; A. beccariana to East Malaysia, Brunei, and Kalimantan of Indonesia; A. hirta to south of Thailand and northeast and south of Peninsular Malaysia including Singapore; A. khasiana to Khasi, Meghalaya, of northeast of India; A. rostrata to Peninsular Malaysia; A. rugosa to Kontum of Vietnam and north of Thailand; A. subintegra to south of Thailand; and A. yunnanensis to Yunnan in China.

Interestingly, the Philippines, situated in the northeast, is the only country within that region having six endemic species: *A. brachyantha* in Cagayan, *A. decemcostata* in Laguna, and *A. parvifolia* in Camarines, all three species being concentrated in the Luzon Island, while *A. apiculata* in Bukidnon and *A. citrinicarpa* and *A. urdanetensis* in Mount Urdaneta, all in Mindanao, the south island. In addition, *A. cumingiana* was also recorded in Mindanao, besides the Maluku Island of neighboring Indonesia. Following the horizontal line, *A. microcarpa* was recorded in Johor, the most southern state in Peninsular Malaysia and in Singapore. It was also found on the Borneo Island, consisting of East Malaysia, Brunei, and Kalimantan of Indonesia.

No	Species names ^a	Basionyms and synonyms	Year first reported	Distribution	Conservation status (IUCN)
1	Aquilaria apiculata Merr.	-	1922	Philippines	-
2	Aquilaria baillonii Pierre ex Lecomte	-	1915	Cambodia	-
3	Aquilaria banaensis P.H.Hô	Aquilaria banaensae	1986	Vietnam	1998: Vulnerable D2
4	Aquilaria beccariana Tiegh.	Aquilaria cumingiana var. parvifolia Aquilaria grandifolia Gyrinops brachyantha Gyrinopsis grandifolia	1893	Brunei Indonesia Malaysia	1998: Vulnerable A1d
5	<i>Aquilaria</i> <i>brachyantha</i> (Merr.) Hallier f.	Gyrinopsis brachyantha	1922	Philippines	-
6	<i>Aquilaria</i> <i>citrinicarpa</i> (Elmer) Hallier f.	Gyrinopsis citrinicarpa	1922	Philippines	-
7	<i>Aquilaria crassna</i> Pierre ex Lecomte	-	1914	Cambodia Laos Thailand Vietnam	1998: Critically Endangered A1cc
8	<i>Aquilaria</i> <i>cumingiana</i> (Decne.) Ridl.	Aquilaria pubescens Decaisnella cumingiana Gyrinopsis cumingiana Gyrinopsis cumingiana var. pubescens Gyrinopsis decemcostata Gyrinopsis pubifolia	1922	Indonesia Philippines	1998: Vulnerable A1d
9	<i>Aquilaria</i> <i>decemcostata</i> Hallier f.	-	1922	Philippines	-
10	<i>Aquilaria filaria</i> (Oken) Merr.	Aquilaria cuminate Aquilaria tomentosa Gyrinopsis acuminate Pittosporum filarium	1950	Indonesia Philippines	-
11	<i>Aquilaria hirta</i> Ridl.	Aquilaria moszkowski	1901	Indonesia Malaysia Singapore	1998: Vulnerable A1d

 Table 1.2
 Accepted names, distribution, and conservation status of Aquilaria species

(continued)