International Journal of Horticulture and Food Science 2020; 2(2): 25-35

E-ISSN: 2663-1067
P-ISSN: 2663-1075
IJHFS 2020; 2(2): 25-35
Received: 18-06-2020
Accepted: 24-07-2020

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Boosting up the economy of Pakistan through agarwood production

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Abstract
Aquilaria species have been the primary source for agarwood and are distributed eastward from India (Bengal and Assam) to the island of New Guinea and southward from tropical China. Agarwood is dark resinous heartwood that forms in Aquilaria and Gyrinops trees in the plant family Thymelaeaceae. Agarwood has been used for medicinal purposes for thousands of years, and continues to be used in Ayurvedic, Tibetan and traditional East Asian medicine. High-grade agarwood powder is used for aromatherapy and also used in the production of pharmaceutical tinctures. The number and types of agarwood metabolite constituents of each reported studies vary depending on the agarwood source, extraction methods and analysis approaches used. Agarwood, also known as eaglewood or gaharu, is a valuable non- timber forest product which sometimes grows in Aquilaria species. The genus species occur mainly in South and Southeast Asia. As a result of a defense mechanism to fend off pathogens, Aquilaria species develop agarwood which can be used for incense, perfume, and traditional medicines. The main markets for these products are in South and East Asia and the Middle East. The high prices demanded for agarwood has led to the rapid depletion of Aquilaria trees in natural forests. The search for agarwood has spread from one country to another. Efforts have been undertaken to increase the production of the infected wood by deliberately wounding the trees. A variety of methods is used towards this end. Some recently developed techniques have proven to be most effective. This resulted in planting of Aquilaria trees by small holders as well as large industrial size plantations. In this paper, we shall discuss all the concerns of agarwood for better yield, quality and maximum utilities for humanity as well as the environment safety to enhance the socio economic condition of the Pakistan.

Keywords: domestication, future developments, plantations, pakistan, socio -economic

Introduction
Aquilaria species have been the primary source for agarwood and are distributed eastward from India (Bengal and Assam) to the island of New Guinea and southward from tropical China. The resin, is widely used for the production of incense and perfume (essential oils) and for medical purposes (Mohamed and Lee, 2016; Rhind, 2014) [65, 91]. The valuable resin develops and accumulates in the stem and branches of the tree after a process of injury and fungal infection (Donovan and Puri, 2004; Rasool and Mohamed, 2016; Sangareswari *et al*., 2016) [36, 90, 95], but this process occurs in only a few trees in wild populations (Paoli *et al*., 2001) [81]. The demand for and trade in agarwood continues today, and with in-creasing wealth in consumer countries over recent decades, demand exceeds supply (Barden *et al*., 2000; Compton and Ishihara, 2006) [15, 32]. This has resulted in increased prices (Wyn and Anak 2010) [116], natural resource decline (Zhang *et al*. 2008) [122], reduction in product quality (Antonopoulou *et al*., 2010) [8], increasing interest in cultivation (Hoang and Nghi, 2011; Page and Awarau, 2012; Persoon, 2008; Rahman *et al*., 2015) [49, 80, 84, 88], and developing methods for resin induction (Liu *et al*., 2013; Mohamed *et al*., 2014; Persoon, 2008) [67, 84, 77]. In Japan, it is known asjinkoh (also means the sinking incense) (Brechbill, 2012) [22]. In European languages such as Portuguese, agarwood is known asaguilaorpaod' aguila; in French, the wood is recognized as d'aigle; and in English as eaglewood. The latter is taken from Medieval Latin (*aquilaria*) which is derived from the Greek (agallochum). The Sanskrit word for incense is known as agar bhatti, which is derived from the word for agarwood (agāru) (McKenna and Hughes, 2014) [72].

Agarwood Manufacturing
Agarwood is dark resinous heartwood that forms in Aquilaria and Gyrinops trees in the plant family Thymelaeaceae (Blanchette, 2006) [18].

It is highly prized that is extremely rare. It has at least a 3000 years history in the Middle East, India, China and Japan (Le, 2003) [64]. In Japan, agar incense is more expensive than gold (Japan Talk, August 26, 2012). The value of agarwood shipped out of Singapore alone each year has been estimated to exceed $ 1.2 billion (Hansen, 2000) [46]. This aromatic resinous wood has many common names including agarwood, gaharu, eaglewood, aloeswood, agila wood, aguru, agar, oud, ude, ud, ood, oode, jinkoh, jinko, Ch'Ing Kuei Hsiang, Ch'En Hsiang, Chan Hsiang, Chi Ku Hsiang, Huang Shu Hsiang, kalambak, grindsanah, etc (Blanchette and Van Beek, 2005) [20]. Agarwood is a diseased tissue corresponding production of oleoresin which begins to become odoriferous and this aromatic resinous wood is called agarwood (Ba, 2010) [12]. At this moment, no other substances are similar as agarwood. Chemical substitutes are already available for perfume but do not come even close in emulating the natural product (Ali, 2006) [7].

The identification of the small proportion of the trees having agar is difficult and destructive, which added greatly to the near-extinction of natural stands of trees (Blanchette, 2006) [18]. Also, large-scale logging operations have destroyed many forested areas where the Aquilaria trees are found. Thus, the current source of agarwood, the naturally-growing old-growth Aquilaria trees is becoming extinct (Hansen, 2000) [46].

Aquilaria malaccensis is a large, evergreen tree, up to 20 - 40 m tall (Adelina, 2004) [2]. This tree has an unusual anatomy and specialized cells within the xylem that produce the resin (Norsuzieana, 2009) [79]. Flowers are hermaphroditic, up to 5 mm long, fragrant and yellowish green or white which blooms at June. Fruits which appear in August are green, egg-shaped capsule, leathery covered with fine hairs, 4 cm long, and 2.5 cm wide. There are two seeds per fruit (Sumarna, 2008) [105].

Aquilaria is unique in producing phloem bundles within the xylem. This network of phloem and parenchyma produce and distribute the resin around affected areas as a tree defense reaction (Blanchette and Van, 2005) [20].

Uses of agarwood

Medicine: Agarwood has been used for medicinal purposes for thousands of years, and continues to be used in Ayurvedic, Tibetan and traditional East Asian medicine. High-grade agarwood powder is used for aromatherapy and also used in the production of pharmaceutical tinctures. It is prescribed in traditional East Asian medicine to promote the flow of qi, relieve pain, arrest vomiting by warming the stomach, and to relieve asthma (Hajar, 2013) [45].

Although, several commercial synthetic agarwood fragrance compounds are available, they can produce only low-quality fragrances, owing to the chemical structure of natural agar oil. Agarwood incenses have also been used as a fragrance in soaps and shampoos (Schippmann, 2001) [96].

Incense: Agarwood incense is burned to produce a pleasant aroma, its use ranging from a general perfume to an element of important religious occasion. It is highly psychoactive and is used for incense (Qi *et al.,* 2005) [87]. Taiwanese consumers purchase agarwood for the manufacture of incense sticks, which are used in prayers during many traditional festivals and ceremonies to bring safety and good luck (TRAFFIC East Asia-Taipei, in litt. to TRAFFIC International 1, 2 May 2000). In Japan, it is considered by many to be sacred and is used to anoint the dead. In Buddhism, it serves as a major ingredient in many incense mixtures, and it is considered to be one of the three integral incenses, together with sandalwood and cloves (Barden *et al.,* 2000) [15].

Economic values

The value of first-grade agarwood is extremely high. It is sold in the form of woodchips, wood pieces, powder, dust, oil, incense ingredients and perfume for several thousand US dollars per kilogram (Barden *et al.,* 2000; Gunn *et al.,* 2004; Compton, 2007) [15, 31] which varies with geographical location and cultural deposition. Agarwood chips start at $30 per kilo up to $9,000 per kilo depending on how much resin is inside the chips (Babatunde, 2015) [13]. First-grade agarwood is one of the most expensive natural raw materials in the world.

Agarwood oil fetches similarly high prices (Agarwood "Wood of Gods" International Conference, 2003). When agarwood chips are processed into oil, the agarwood oil was sold at US $ 30,000 per kg (Nanyang Siang Pau, 15 August 2005). The current global market for Oud oil and other related agarwood products is estimated to be in the range of US$ 6 to 8 billion (Akter *et al.,* 2013) [5] and the major industry buyer of Oud oil, is expected to exceed US $ 36 billion in 2017. For Integrated cropping - Set up 1,000 ha plantation for agarwood and banana production internal rate of return (IRR) and benefit-cost ratio (B/C ratio) are 54.85% and >1 (3.30) respectively (Mamat *et al.,* 2010) [70].

There are about 100 enterprises producing agar wood and agar oil in Bangladesh (Abdin, 2014) [1]. Local entrepreneurs are claiming that this is a 100% export oriented sector based on local raw materials and using indigenous technology. They are exporting about US$ 62.5 to 125 thousand per year.

The agar tree (*Aquilaria malaccensis*) contains dark resinous heartwood called agarwood, eaglewood, oud, gaharu or aloeswood (Barden *et al*., 2000) [15], and offers a primary source of essential oil called oleoresin. This wood has high demand for medicine, incense and perfumes across Asia, Middle East and Europe (Abdin, 2014) [1].

In Bangladesh, the production of agar-wood started about 400 years ago in the Suzanagar union under Barlekha Upazila of Moulvibazar District in Sylhet (Abdin, 2014) [1]. About 25,000-30,000 workers were engaged in cultivation, collection, processing and marketing of agar and agar-based products in the country (Baksha, 2009) [14]. However, little is known about the economic feasibility of agar production in Bangladesh. Reliable information on the financial performance of agar plantations is also lacking (Rahman *et al.,* 2014; Uddin *et al*., 2018; Akter *et al*., 2008; Islam *et al*., 2014; Akter *et al*., 2013) [110, 4, 5].

Economic Benefits

Agarwood is a precious incense substance which is extremely rare. This fragrant wood is only formed in Aquilaria and Gringos species under family the Thymelaeaceae (Blanchette, 2006) [18]. The more resin deposit, the more quality ensure and the precious wood would be formed. It has been using for 3000 years in the China, Japan, India, and especially in Arabian countries (Le, 2003) [64] and known by many names depending on place and cultures such as Agar (Bangladesh, India), Aguru

(Sanskrit), Oud (Arabic), Eagle/Agilawood (Europe) (Akter *et al*., 2013) [5].

By 1970s, it was sold up to $42.5 and then raised straightly $1250 and $2500 per kg in 2000 and 2005, respectively (Abdin, 2014) [1]. First-grade agarwood is one of the most expensive natural raw materials in the world. Singapore trades agarwood $1.2 billion alone each year (Hansen, 2000) [46]. The trading value of pure agar oil is $30,000 to 40,000 per kilogram (Abdin, 2014) [1]. Based on resin presence, agarwood chips sell from $30 to $10,000 per kilogram (Babatunde, 2015) [13]. Akter *et al*., (2013) [5] reported that in the year of 2013, the global market for agar oil another agarwood related products was estimated in the range of $6 to 8 billion and the major agar oil industrial buyer expects to exceed it up to $36 billion in 2017 (http://www.ouddh. com/?cid =2065877).

Agarwood formation is a result of resin synthesis in the xylem tissues of Aquilaria plant. This resin is a chemical substance which consists of aromatic, sesquiterpenes, fatty acids, chromones, phenolic and especially 3-phenyl-2-butanone and alpha-cubebene (Naef, 2011; Hashim *et al*., 2014) [78, 47]. Artificial agarwood or agar oil formation is quite impossible as it is a composition of numerous volatile and semi-volatile compounds. However, the chemical composition of cultivated agarwood is almost similar with naturally inoculated agarwood (Espinoza *et al*., 2014) [37].

Aquilaria spp. trees are indiscriminately and excessively harvested within all ecological zones, and *Aquilaria crassna* is categorised as ‘Critically Endangered’ by IUCN (Hilton-Taylor, 2000) [48]. Aquilaria trees grow well under optimal conditions and annual growth of over 1 m in height per year is common. For further technical information, reference is made to Species Monograph #12 produced by the Lao Tree Seed Project (Jensen, 2002) [56].

The agarwood producing tree species are found from India eastwards to Southeast Asian countries (such as Vietnam, Thailand, Malaysia, and Indonesia) to the island of New Guinea (Zich and Compton, 2002) [124]. The production of agarwood is uncertain and it is estimated that only 10% of the Aquilaria trees in the forest may contain agarwood (Donovan and Puri, 2004) [36].

In the Middle East, agarwood is a symbol of status, wealth and hospitality. An important use of agarwood is the production of incense, the most prized of all incenses. In Middle East, oudh (Arabic word for agarwood) is burnt to release the unique scent and it is a symbol of status, wealth and hospitality (Chang *et al*.2002) [23]. Agarwood is also used as an ingredient in Ayurvedic, Tibetan, Chinese and other traditional Asian medicine. It is also used in the perfume and toiletry products such as soap and shampoo (Chang *et al.*, 2002) [23].

Uses of Agarwood Use

	Use	**Remark**
Production of Incense	Homes, religious ceremonies, rituals and meditation	Precious incense
Oud (Arabic word for wood)	Symbol of status, wealth and hospitality in Middle East	-
Gaharu chips	homes in Middle East	-
Gaharu oil	in perfume and toiletry products such as soap & shampoo	-
Wood	Ayurvedic, Chinese and other Asian traditional Asian medicine	
Wood	sculptures, beads & boxes	Religious purpose

Source: Chang *et al.*, 2002 [23] and Barden *et al.*, 2000 [15]

Agarwood is a resin filled wood produced in Aquilaria and Gyrinopstrees with a long history of use as incense, perfume and in traditional medicine. Agarwood, has many different names depending on the region of the world where it is found including agar, aloes wood, eagle wood, jinkoh, oud, gaharu, Ch’en Hsiang and Chen-Xiang (Antonopoulou *et al*., 2010, CITES 2005, Compton and Ishihara, 2004) [8, 33, 29].

The loss of this tree from the landscape has been of great concern and several Aquilaria species have been considered endangered according to the International Union for the Conservation of Nature which lists it on their Red List of Threatened Species (IUCN, 2013) [53].

Uses for agarwood and its value: Agarwood is used throughout the world but there are two major regions of consumption, the Middle East and Asian markets (Antonopoulou *et al*., 2010, CITES 2005, Compton and Ishihara, 2004) [8, 33, 29].

An essential oil distilled from agarwood has a very long history of use as perfume. Known in the Middle East as oudh (or oud) it canbe used as a pure oil or blended with various other substances. This essential oil is mentioned in the bible several times as “aloes” wood (Musselman 2007). There are also descriptions of it use in ancient Islamic religious documents and in reports of its extensive use as perfume as early as the 13thcentury (Hansen, 2000) [46].

Agarwood has also been used in traditional medicines. It has been reported as a component of many traditional Ayurvedic remedies in the Indian subcontinent as well as being used in Tibetan, Chinese, Malayan and Vietnamese medicine (Barden *et al*., 2000, Kiet 2003 [15, 61], Lim and Anack 2010) [15]. More modern studies confirm that agarwood has bioactive products that function as effective anti-microbial compounds, it may have anticancer activity, can be used as an antidepressant and used to promote good health in general (CITES, 2005, Dash *et al*., 2008, Mei *et al*., 2008) [29, 35, 74].

Field trials over many years using methods that mimic the natural production of agarwood have shown great success and are now being used to produce high quality agarwood in young plantation grown trees in many areas of the Indomalaya region (Blanchette and van Beek, 2005) [20].

The resin is chemically complex and consists of sesquiterpenes, chromones, fatty acids and phenolic compounds (Naef 2011, Mei *et al*., 2010) [73, 78]. As many as 70 terpenoids have been identified and more continue to be characterized (Espinoza *et al*., 2014) [37].

Agarwood is a dark coloured, fragrant resin embedded in the otherwise whitish wood of Aquillaraia species. It is accumulated in the roots and the trunk of the tree as nodules of varying age, shape, size and commercial quality, and is formed as a result of injury and fungal infection (Blanchette, 2007; Jensen, 2009) [19, 56]. Agarwood product is very common in many religious rituals. Wooden pieces and sculptures, beads and bracelets are highly appreciated goods in South and Southeast Asia. However, the major use of agar wood is in the form of extracted essential oil as perfumes, other cosmetic products and in the incense industry. Agar oil is also used in traditional medicines and wine in China and Korea and in ayurvedic medicine in India (Barden *et al*., 2000; Chen *et al*., 2011; Gunn *et al*., 2003; Persoon, 2007) [15, 25, 44, 83]. The popularity of Agarwood products in the international market offers the opportunity for a high earning livelihood.

It was evident from the reconnaissance survey and interaction with the villagers that BKS had all the capital (natural, social, human, physical and financial) as household wealth in varying degrees to pursue different livelihood activities (Sherbinin *et al*.,2008) [98].

Agarwood products were seen as an important strategy to earn a high income by virtue of being expensive and therefore, many villagers were attracted to this livelihood activity (Persoon, 2007) [83].

Combining plantation or farm activities with wage labour provided a temporary financial relief especially in times when the demand for cash was high for example, paying school fees and expenditure of customary ceremonies (Koczberski *et al*., 2001) [63].

Trees and native vegetation improve the soil beneath them as organic matter, nitrogen and other nutrient content are comparatively higher (Roy *et al*., 2010) [94] in the plantation area. The soil sample data in the present study was not adequate enough to clearly indicate the trend in tree covered land versus annual cropping land as nitrogen, phosphorus and potassium level was similar in both types of land use. Young age plantations are reported to have no impact on phosphorus content of the soil (Zeng *et al*., 2010) [121]. However, it was indicated in the present study that the pH level was less acidic in tree covered land as compared to cassava and maize cultivated land.

Agarwood is a highly prized non-timber forest product which can be used in fragrances, incense, medicines, aromatherapy and religious ceremonies (CITES, 2004; 2005) [30]. The value of agarwood shipped out of Singapore alone each year has been estimated to exceed $1.2 billion (Hansen, 2000) [46]. The most important source of agarwood is the Aquilaria spp. tree, which is an angiosperm within the Thymelaeaceae family (Rogers, 2009) [93].

In fact, Hong Kong is literally named the “Fragrant Harbour” as it was one of the significant trading centers for agarwood and incense in the early days, when Aquilaria sinensis was widely traded in Hong Kong and then transported to numerous Chinese provinces, Southeast Asia as well as Arabia, along with other incense and spices (Fu, 2008) [39].

The healthy wood of Aquilaria trees is white, soft and without scented resins. In a natural environment, agarwood forms only when affected by certain external factors, such as lightning strike, animal grazing, insect attack or microbial invasion, typically around wounded or rotting parts of the trunk (Blanchette and Heuveling, 2009; Pojanagaroon, and Kaewrak, 2006) [21, 85].

Agarwood formation occurs slowly and infrequently in old trees and the supply of agarwood from wild sources is far less than market demand. Because of its immense value and rarity, indiscriminate cutting of trees and overharvesting in hope of finding the treasured resin has led to the depletion of wild trees (Zhang *et al*., 2008; Soehartono *et al*., 2000) [122, 101]. Eight Aquilaria species, including A. sinensis, were listed on the IUCN red list as endangered species (IUCN, 2008) [51].

Gonystylus genus has 20 species, scattering in Southeast Asia regions until Solomon and Nicobar archipelago. *Gyrinops* genus has seven species. Six out of seven are found in East part of Indonesia and one species encountered in Srilanka (Aswandi, 2009) [9].

The result showed that their relative population in Sumatera, Kalimantan, Nusa Tenggara, Sulawesi, Maluku and Papua was 26, 27, 5, 4, 6 and 37% respectively (Adijaya, 2009) [3].

In order to anticipate the increasing demand and to evade agarwood in nature to become extinct, several efforts to cultivate trees producing agarwood have started to develop in many areas of Indonesia such as North Sumatera, Riau, Jambi, West Java, West Nusa Tenggara, South Kalimantan and West Kalimantan. Agarwood cultivation that is increasing especially after conducting several researches show that cultivated agarwood could provide feasible benefit (Marliani, 2008; Suharti, 2009; Tarmiji, 2009; The Angel, 2009) [71, 104, 108].

Agarwood exerts an important role in Indonesia as it contributes to country's foreign exchange. High economic value of agarwood has induced agarwood to become one of the prominent commodities in Indonesia (Pratiwi, 2010) [86].

Agarwood trees will not grow well on inundated soil, swamp area, soil solum thickness less than 50 cm, and quartz sand as well as soil with the acidity levelless than 4 (Rizlani and Aswandi, 2009) [9]. Harvesting could be done before the trees die; however, agarwood is best harvested on the dead trees because three types of agarwood products: and ash/powder could be obtained all at once (Sumarna, 2007) [106].

Agarwood incenses have also been used as a fragrance in soaps and shampoos (Schippmann 2001) [96]. It is prescribed in traditional East Asian medicine to promote the flow of qi, relieve pain, arrest vomiting by warming the stomach, and to relieve asthma (Hajar, 2013) [45]. It is sold in the form of woodchips, wood pieces, powder, dust, oil, incense ingredients and perfume for several thousand US dollars per kilogram (Barden *et al*., 2000, Gunn *et al*., 2003 [15, 44], Compton, 2007) [31]. Agarwood chips start at US$30 per kg up to $9,000 per kg depending on its resin percentage (Babatunde, 2015) [13]. Agarwood oil fetches similarly high prices (Agarwood “Wood of Gods” International Conference 2003). The highest grade agar oil is produced in China and is reported to cost over Tk.0.9 million (US$11,500) per kg. The second-best quality agar oil is sourced from Vietnam (Rahman *et al*., 2015) [88].

A whole range of qualities and products are on the market, varying in quality with geographical location, botanical species, and the age of the specific tree, inoculation method and the section of the tree where the piece of agarwood stems from (Jung, 2011) [58]. The value of agarwood shipped out of Singapore alone each year has been estimated to exceed US$1.2 billion (Hansen 2000) [46]. The current global market for agar oil and other related agarwood products is estimated to be in the range of US$6 to 8 billion (Akter *et al*., 2013) [5].

In Bangladesh, Aquilaria malaccensis (Alam, 2004), Aquilaria agallocha (Bhuiyan *et al*. 2009 and Rahman *et al*.2015) [17, 88] are commonly cultivated. Agar trees were planted in monoculture and block plantations. 75% of the plantations have been established on degraded land as well as denuded hills and rest 25% were established at the homesteads of farmers (Rahman *et al*., 2015) [88].

After 3 to 5 years of nailing, the trees became ready for harvesting and the best time for harvesting is mid-October to mid-March (Chowdhury *et al*., 2016 and Rahman *et al*., 2015) [27, 88].

On an average, every enterprise uses 600 kg to 4 metric tons agarwood for producing 0.74-5.75 kg agar oil where production cost and total returns are BDT0.5 million and BDT1.3 million, respectively (Rahman *et al*., 2015) [88] with a net profit of BDT0.8 million.

Abdin tried to find out some barriers toward the development of Bangladesh agarwood industry (Abdin 2014) [1]. Due to lack of contemporary price information of different markets, forest-based enterprises do not receive high prices for their products (Rahman *et al*., 2009) [89].

Agarwood has been widely used as therapeutic perfumes, traditional medicine, religious purposes and aromatic food ingredient (Liu *et al*., 2013) [67]. Some of the earliest known uses of agarwood were recorded in ancient literatures, religious scriptures and medical texts. The word "aloes" which means agarwood was found occurring in the Sanskrit poet, Kâlidâsa that can be dated back to 4^{th}–5^{th} century (Lee and Mohamed, 2016) [65]. Meanwhile, the use of agarwood in the prescription of traditional Chinese medicine of the same period had also been recorded. The Chinese medicine uses it as a natural sedative, pain reliever, digestive aid and carminative (Ye *et al*., 2016).

Agarwood has high demand throughout the world as a raw material for incense, perfume and medicine purposes, with Middle East and East Asia as the two major regions of consumption (Antonopoulou *et al*., 2010) [8]. As the wealth of the consumer countries has gradually increased in the recent decades, the market's demand for agarwood started to exceed its supply.

Global agarwood prices can be ranging from US$ 20 – 6,000 per kilogram for the wood chips depending on its quality or US$ 10,000 per kilogram for the wood itself (Abdin, 2014) [1]. In addition, the value of agarwood essential oil can be as high as US$ 30,000 per kilogram. The annual global market for agarwood has been estimated to be in the range of US$ 6 – 8 billion (Akter *et al*., 2013) [5], yet a large number of the trades have not been recorded.

Aquilaria belongs to the Thymelaeaceae family of angiosperms, which is endemic to the Indomalayan realm. To date, there is a total of 21 Aquilaria species which have been documented and 13 of them are recognized as the agarwood-producing species (Lee and Mohamed, 2016) [65]. The destructive exploitation of agarwood, however, has badly affected the wild population of all Aquilaria species. As a consequence, the genus is now listed as endangered species and protected under Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) regulation due to a drastic declination of the species in the wild (Convention On International Trade In Endangered Species (CITES, 2004; Lee and Mohamed, 2016) [65, 29]. High demand of quality agarwood in conjunction with the depletion of the wild Aquilaria trees implied that the price of the agarwood will continue to soar. As an alternative, mass cultivation and large plantation of Aquilaria trees which serve as a sustainable source to obtain agarwood have greatly resolved the shortage of agarwood supply in the global market.

Since healthy Aquilaria tree does not form agarwood, leaving it worth next to nothing, the scarcity of naturally occurring agarwood has prompted the development of artificial agarwood-inducing methods. Efforts to artificially induce the agarwood formation can be traced back to as early as 300C.E. in the Chinese history, where it was recorded that resin deposition accompanied with color changes of internal tissues can happen within a year by injuring the trees (López-Sampson and Page, 2018) [68]. Besides mechanical wounding approach, the use of chemical, insect and pathogen-inducing techniques is increasingly common in the agarwood industry nowadays (Mohamed *et al*., 2014; Kalita, 2015) [77]. All of these induction techniques in any case mimic the natural processes of agarwood formation, which have their own strengths and weaknesses. In this article, we endeavor to provide a more comprehensive coverage of existing induction methods and their development prospects using the advancement of biotechnology. To better understand the agarwood formation process, the molecular mechanism of secondary metabolite biosynthetic pathways underlying the resin production will also be elaborated. The indiscriminate harvesting of agarwood from natural habitats has seriously hampered natural regeneration of Aquilaria trees, thus threatening the survival of the species in the wild. In order to meet the high market demand yet to protect the species from extinction, mass plantations of Aquilaria trees have been established across the Asian countries to allow sustainable agarwood production (Azren *et al*., 2018). Since agarwood formation in natural environment is a very long process which can take up to 10 years, the development of effective induction technology has received a great attention as it is extremely crucial to ensure the stability of agarwood yield from the domesticated Aquilaria trees.

Naturally, agarwood formation is often linked to the physical wounding or damage of Aquilaria trees caused by thunder strike, animal grazing, pest and disease infestations (Rasool and Mohamed, 2016; Wu *et al*., 2017) [90, 115]. These events expose the inner part of the trees toward pathogenic microbes, which elicit the defense mechanism of Aquilaria to initiate the resin production. This natural formation process of agarwood has greatly inspired the development of diverse artificial induction methods (Table1). For example, many traditional induction approaches like nail in setting, holing, burning, trunk breaking and bark removal have adopted the concept of physically wound the trees (Mohamed *et al*., 2010; Azren *et al*., 2018). Although it is cost effective and requires only personnel with little or no scientific knowledge on agarwood, but these induction methods usually result in inferior quality and uncertain yield of agarwood.

With more understanding on Aquilaria-fungal interactions in promoting the agarwood formation, the induction methods gradually shifted from sole mechanical wounding into deliberate wounding coupled with the application of biological inoculums (Jong *et al*., 2014) [57]. Many pure-culture strains of fungi isolated from natural agarwood were found to be effective biological agents to induce agarwood formation in healthy Aquilaria trees (Cui *et al*., 2013; Siburian *et al*., 2015; Sangareswari *et al*., 2016) [34, 99, 95]. The fungal infected Aquilaria trees were reported to deposit agarwood resin around the infected sites as barrier to prevent further fungal intrusion (Cui *et al*., 2013; Rasool and Mohamed, 2016) [34, 90]. One obvious advantage of using fungal inoculum is that it is generally believed to be safe for handling and eco-friendly. However, fungal inoculation will normally give rise to localized and inconsistent quality of agarwood due to the different fungal consortium used. As a solution, laborious holing process and long incubation time is required to maximize the colonized surface area on the tree to produce better quality of agarwood (Mohamed *et al*., 2014) [77].

Aquilaria trees to initiate agarwood resin biosynthesis pathways (Liu *et al*., 2013; Wu *et al*., 2017) [67, 115]. Chemical inducers normally comprise of phytohormones, salts, minerals and biological derived substances (Zhang *et al*., 2012; Liu *et al*., 2013; Van *et al*., 2015) [67, 123]. Besides, suitable delivery method is often developed together with the chemical formulations to ease the large-scale induction process, such as vessel equipped with transfusion needle (Yang *et al*., 2014c). To date, several induction approaches have been developed based on the chemical induction concept such as cultivated agarwood kit (CA-kit), the whole-tree agarwood inducing technique (Agar-Wit) and biologically agarwood-inducing technique (Agar-bit). CA-kit is a combined method based on physical wounding and chemical induction, where the inducing agent is applied into the Aquilaria tree via an aeration device inserted into the wound (Blanchette and Heuveling, 2009) [21]. This method results in satisfying yield and quality, but the procedures are in some way conventional. On the other hands, Agar-Wit is a transpiration-assisted chemical treatment to form an overall wound in the tree, where the preloaded inducer in a transfusion set is distributed via plan transpiration (Liu Y. *et al*., 2013) [67]. Through this method, a larger agarwood coverage area can be achieved, but unfortunately produces more decayed tissues. Similarly, Agar-bit method adopts the idea of distributing the inducing reagent by plant transpiration, except that the reagents are injected directly into the stems of the tree (Wu *et al*., 2017).

Through chemical induction approach, the time-consuming holing process can be minimized as less induction sites are needed to deliver the inducers throughout the plants via transpiration process. Properly formulated inducer was shown to be able to produce artificial agarwood with quality closely resembled to those obtained from natural source (Liu *et al*., 2013) [67]. In spite of the fast results and high yields, the application of chemical inducers still poses skepticism of toxicity on both human and environment. The currently adopted agarwood quality assessment in the market has been extensively reviewed by Liu *et al*. (2017). Recently, the metabolite analysis of agarwood has gained increasing attention as some studies showed that there is correlation of agarwood quality to its resin yield and metabolite constituents (Pasaribu *et al*., 2015; Liu *et al*., 2017). Many studies have been conducted to clarify the metabolite composition of agarwood obtained either from wild or artificially induced methods (Chen *et al*., 2012; Gao X. *et al*., 2014; Hashim *et al*., 2014) [24, 47]. It was concluded that the composition of agarwood resin is mainly composed of the mixtures of sesquiterpenes and 2-(2-phenylethyl) chromones (PECs) (Naef, 2011; Chen *et al*., 2012; Subasinghe and Hettiarachchi, 2015) [24, 78].

Meanwhile, the constituents of agarwood essential oil were shown primarily to be sesquiterpenoids (Fazila and Halim, 2012; Hashim *et al*., 2014; Jayachandran *et al*., 2014) [38, 47, 54]. Together, all of these major compounds and some low abundant volatile aromatic metabolites form the unique and fragrant-smelling property of agarwood.

The number and types of agarwood metabolite constituents of each reported studies vary depending on the agarwood source, extraction methods and analysis approaches used (Fazila and Halim, 2012; Jong *et al*., 2014; Pasaribu *et al*., 2015) [38, 57]. Nonetheless, there are over 150 compounds as reviewed by Naef (2011) [78] have been identified thus far in agarwood from different sources.

The better insight of agarwood metabolites will definitely facilitate the identification of universally accepted biomarkers for agarwood grading. Since the publication of the comprehensive review of Naef (2011) [78] regarding the major constituents of agarwood, new compounds continue to be discovered in the later studies (Wu *et al*., 2012a; Yang *et al*., 2014b; Wang *et al*., 2015). The number of discovered compounds in agarwood will certainly be further increased in the future.

Agarwood formation can be related to the self-defense mechanism of Aquilaria trees in response to (Gao *et al*., 2012b; Singh and Sharma, 2015).

Due to the rampant destruction of natural habitats, most agarwood-bearing species have been relegated to the status of endangered species. In fact, all 19 known Aquilaria species are included under CITES (CITES, UNEP-WCMC Species Database: CITES-Listed Species, 2019) and the Red List of the IUCN (IUCN Red List of Threatened Species, 2019) [52].

In a recent study by Chen *et al*. (2018) [26], a fungus, Rigidoporus vinctus, was isolated from the inner layer of infected A. sinensis trees.

It was found that the Agar-Bit method up regulated the expression of some biological synthase genes (farnesyldiphosphate synthase, sesquiterpene synthase, and chalcone synthase) compared to the mechanically stimulated agarwood (Wu *et al*., 2017; Tan *et al*., 2019). Subsequently, Subasinghe *et al*. (2018) carried out an artificial induction by Aspergillus nigerand F. solanifor agarwood resin formation in Gyrinops wallatrees.

Genetic engineering of plants has been used to produce terpenes by successfully expressing heterologous terpene synthase genes to produce novel monoterpenes, sesquiterpenes, and diterpenes (Besumbes *et al*., 2004; Lucker *et al*., 2004; Wu *et al*., 2006) [114]. In one of the cited examples, Wu *et al*. (2006) [114] used the patchoulol synthase gene from patchouli to transform tobacco.

Trichome-specific promoters can be used to drive the accumulation of the terpenes specifically into leaf trichomes, which can confer ease of recovery of the compounds (Kim *et al*., 2008; Shangguan *et al*., 2008).

In a recent study, cloning of two new genes, AmSesTPS1and AmGuaiS1, from *A. malaccensis* has been reported (Azzarina *et al*., 2016).

Jasmonate induces expression of sesquiterpene synthase ASS1, which is a major enzyme in the biosynthesis of agarwood sesquiterpenes in A. sinensis (Xu *et al*., 2017).

In a subsequent study, Kumeta and Ito (2010) further enhanced the understanding of agarwood biosynthetic machinery by revealing the genomic organization of the δ-guaiene synthase gene in *A. crassna*.

In a study by Kenmotsu *et al*. (2011), it was found that the biosynthesis of spirovetivane-type sesquiterpene in *A. microcarpais* triggered by the treatment with methyl jasmonate due to enhanced expression of farnesyl diphosphate synthase, calmodulin, and Rac/Rop GTPase

genes.
Tubulin, ribosomal protein, and glyceraldehyde 3-phosphate dehydrogenase genes were identified as the most stable reference genes for quantification of target gene expression in A. sinensis in the future (Gao *et al*., 2012a).
Gao *et al*. (2012b) discovered 27 novel miRNAs in A. sinensis, and expression levels of 10 stress-responsive miRNAs were correlated with wounding. Eight of them were wound-responsive, which shows that the existence of miRNAs is indicative of their critical role in stress reactions, leading to agar formation (Gao *et al*., 2012b).

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