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REVIEW

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Fungi mediated agarwood (*A. malaccensis*) production and their pharmaceutical applications: A systematic review

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ABSTRACT

Aquilaria malaccensis is a tropical tree that produces expensive resinous heartwood agarwood through the natural process induced by natural or artificial injury or microbial infection. Fungi are commonly noticed as the main microbial component responsible for agarwood formation. The current review investigated the agarwood quality and fungi diversity in artificial and natural agarwood from *A. malecensis* trees from the rainforest for various pharmaceutical applications. Apart from being an aromatherapy material, in medicinal prospect, agarwood can be used as a carminative, stimulant for heart palpitation, tonic during pregnancy, remedy during the post-natal recovery period, and cure for the disease of the female genital part. The whitebark of agarwood is believed to heal jaundice and body pain. Moreover, agarwood helps to relieve body pain, warm the abdomen, relieve asthma, treat coughs, and acroparalysis acts as antihistamine, analgesic and anti-inflammatory. In Chinese traditional medicine, it is helpful as a sedative to relieve gastric problems, relieve rheumatism and high fever. Agarwood properties were able to fight cancer cells but very little is known about this process. The present review also illustrated the variety of kinds of pharmaceutical applications in the treatment of various diseases. Current review findings proved that artificial agarwood may produce quality equal to natural agarwood and may not be affected by fungi interacting with the tree, which can be used as a superior pharmaceutical application.

1. Introduction

Malaysia's rainforest is one of the countries rich in its biodiversity and hosts over 500.000 plant species that have been exploited since ancient times for their high economic value (Elias et al., 2017). *Aquilaria* species or *Karas* trees come from the Thymelaeaceae family and are well distributed throughout peninsular Malaysia. This plant species is widely known for its gaharu; a scented wood or agarwood/sandalwood. Agarwood, the fragrant resin-infused wood derived from the wounded trees of *Aquilaria* species, is categorized as a non-timber product and is highly valued in West Asia and the Middle East (Zhang et al., 2010; Ramli et al., 2022). *Aquilaria malaccensis* is the most popular source of agarwood among the *Aquilaria* species and has become the primary agarwood producer in Malaysia. Agarwood contains a unique property by emitting a wonderful fragrant when this dark resinous wood is burnt. As a result, agarwood is widely utilized as an essential component in perfumes and incenses production. In addition, it has also been used in traditional medicines for centuries. It recently has been incorporated into pharmaceutical products to treat many illnesses, including coughs, acroparalysis, and asthma, and has been used as an antihistamine (Mohamed et al., 2014).

Agarwood's high demand and selling price have seriously affected the natural sources of *A. malaccensis* resulting from inappropriate harvesting. Currently, *A. malaccensis* is listed as an endangered species in the Convention on International Trade in Endangered Species of Wild Fauna and Flora. Growing agarwood using an optimum and sustainable method would permit the increasing world market demand for medicines, perfumes, art-crafts, and others to be met. Nowadays, *Aquilaria* trees are being cultivated on a large scale, and many investigations have been performed into

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formulating the best artificial inoculation methods for agarwood induction in plantation trees (Bhuiyan et al., 2009; Mohamed et al., 2014). Both forms of reforestation of *A. malaccensis* and artificial agarwood-inducing methods will guarantee a continuous supply of agarwood and can conserve the wild *A. malaccensis* species.

In natural rainforests, agarwood formation in *A. malaccensis* happens randomly and usually at a low level. The analysis predicted that the trees which develop agarwood due to natural infections only range from 7-10% (Ng et al., 1997). Generally, fungi are considered the significant microbial component responsible for agarwood formation. From the observation, healthy trees never produce fragrant resin. Agarwood formation is mainly attributed to trees' biotic and abiotic stress exposure (which can be physically or chemically), which activates their defense mechanism (Novriyanti et al., 2010). Fungi identification in natural agarwood has been investigated previously, and several species were discovered to be either endophyte or pathogenic. It is known that wounding and microbial infection effectively produce resin in agarwood. However, none of the fungi found was proven to induce artificial agarwood to produce quality similar to nature agarwood. Perhaps, a different fungi community makes the difference. To solve the intersection of fungi species works in nature, agarwood and artificial agarwood would be a tremendous success to ensure continuous superior agarwood supply.

Continuous demand and shortage supply of natural agarwood lead to the production of artificial agarwood. However, the quality of artificial agarwood is arguable as it is not even close to natural agarwood quality. The mechanism of high-quality agarwood formation remains a mystery despite fungal discovery from agarwood samples. Fungal interaction proves a relationship in inducing resin synthesis in agarwood trees. Various species of fungi have been discovered from agarwood and even applied as inoculants, but the agarwood is either low quality or inconsistent. The difference between natural and artificial agarwood may be caused by the different diversity of fungi in both agarwood trees. Thus, comparing these two samples regarding agarwood quality and fungi diversity is necessary to determine an effective fungi group as agarwood inducers. Moreover, the comparison may prove if artificial quality can be equal to nature agarwood based on fungal infection. This review analyses agarwood quality samples from natural and artificial agarwood followed by fungal identification based on morphology observation and molecular approach for future pharmaceutical applications.

2. *Aquilaria* species

Aquilaria spp. and Gyrinops from the botanical family Thymelaeaceae naturally produce distinct fragrance trees. *Aquilaria* spp. is common in tropical climates, as in Asian countries and Papua Guinea. However, not all *Aquilaria* spp. produce resinous wood (Sumarna, 2005; Wiriadinata et al., 2010). *Aquilaria* spp. is distributed from Southeast Asia to China, India, and Papua (New Guinea). Each country has different species of *Aquilaria*. *Aquilaria* spp. prefers to grow in the dipterocarp or mixed dipterocarp forest. It is usually populated at up to 1000 m sea level and from 270 m to 500 m within the temperature of 22 °C to 22 °C (Chua, 2008). However, it may adapt to the plantation of rocky, sandy, or calcareous soil areas equipped with well-drained slopes and ridges (Chakrabarti et al., 1994; Sumadiwangsa, 1997). *Aquilaria* species such as *A. malaccensis*, *A. cumingiana* and *A. microcarpa* were common in Malaysia and Indonesia. Meanwhile, *A. crassna* is found in Malaysia, Thailand, Cambodia, and Vietnam. *A. hirta*, can be found in the forests of Malaysia, Indonesia and Thailand. *A. achalloga* and *A. khasiana* can only be found in India. *A. acuminata* is known in Indonesia, Philippines, and Papua (New Guinea). *A. baillonil* is native to Thailand and Cambodia. Phillipine and Papua (New Guinea) both own *A. filaria* species. Some *Aquilaria* species are endemic in certain countries, such as *A. brachyantha* and *A. rostrata* which are endemic to Malaysia, while *A. sinensis* can only be found in the China region, *A. beccariana* is grown in Indonesia, *A. apiculina* in Phillipine and *A. baneonsis* in Vietnam (Akter et al., 2013).

2.1. Aquilaria malaccensis

A. malaccensis is a tree of about 20-40 m in height and diameter at breast height (dbh) of 60 cm (Duriyaprapan et al., 2003). *A. malaccensis* is a non-timber that appears white, light in weight and soft in density. Leaves of *A. malaccensis* are alternate and elliptic, about 3-5 cm wide and 6-10 cm long, with 12-16 pairs of veins. *Aquilaria* tree would grow for about a hundred years if undisturbed in the forest and start to produce small, white flowers as early as four years old, depending on the species (Akter et al., 2013), but only able to produce seeds between seven to nine years old (Chua, 2008). Its inflorescence is described as a terminal or axillary umbel, containing flowers 5 mm long, yellowish green or white (Duriyaprapan et al., 2003). The juvenile fruit is a green egg-shaped capsule 4 cm long and 2.5 cm wide, with a pubescent leathery exocarp. The mature fruits are blackish brown and can be collected directly from the tree, each containing two seeds (Duriyaprapan et al., 2003). The juvenile fruit of the *Aquilaria* tree appears in an oval shape of 4 cm in length and 2.5 cm in width with a hairy surface. Each fruit contains two seeds (Duriyaprapan et al., 2003). In the forest, the seeds will casted around the mother tree. Seed viability is within one week after seeding mature. Seed germination takes 16 to 63 days, depending on the sowing time after seed maturity. Germination is at the highest rate if the seed is sown immediately (Ng et al., 1997). This may be due to changes in enzymes and hormone accumulation in the seed that control the germination process. Although its fruit is recalcitrant without human interference, seedlings found in the forest hardly grow on commercial plantations after being transplanted. Mature trees can grow up to 40 m in height and reach 2.5 cm dbh with a growth rate of approximately 0 to 1.95 cm per year (Sumadiwangsa, 1997).

2.2. Agarwood production in A. malaccensis

Agarwood production from *A. malaccensis* has been recorded through the discovery of aromatic compounds or terpene family compounds. Wu et al. (2012) found four new sesquiterpenoids and new 2-(2-phenylethyl)-4H-chromone-4-one [-2-(2-phenylethyl)-4H-1-benzopyran-4-one] derivative from agarwood sample of *A. malaccensis*. While mature *A. malaccensis* can produce agarwood, the young tree has been proven to synthesize agarwood compounds such as benzylacetone, anisylacetone, guaiene, and palustrol when infected with fungus (Mohamed et al., 2014). In addition, matured agarwood from wild habitat may produce agarospirol, alloalromadendrene α-elemol, γ-eudesmol, and guaiol when analyzed with gas chromatography-mass spectrometry (GC-MS) (Tsan and Mohamed, 2014). Many previous studies showed that *A. malaccensis* could produce aromatic agarwood when burned or analyzed with a spectrometry instrument (Chen et al., 2011; Jayachandran et al., 2014; Mei et al., 2013).

3. Agarwood

Agarwood refers to aromatic oleoresin resulting from pathological activity in the natural wood of Thymeleacea and Gyrinops. When heated, this oleoresin has unique fragrance properties, making it in demand in the perfumery industry (Ismail et al., 2016; Jayachandran et al., 2014). Resinous agarwood emits a unique soft fragrance when burnt, but its scent is easily detected in super quality agarwood. Distributed in different countries, agarwood is called with a different language and usages relevant to local culture. In Malaysia and Indonesia, agarwood is known as 'gaharu' or 'karas' (Chua, 2008). For the Arabic community who consumes agarwood as raw material in perfumery, they name gaharu as 'oud' (Jung, 2013). In China, agarwood is familiar as 'chen xiang', while in Hindi as 'agar' (Akter et al., 2013). In Vietnam, native people named agarwood 'tram huóng' (Nguyen, 2014). In Japan, agarwood is known as jinkō (Jung, 2013). While vernacular name sound confusing sometimes, their commercial name is widely known as agarwood, aloeswood and eaglewood (Chua, 2008). Quality of agarwood is related to plant species, human engagement, microorganism interaction and some other abiotic factors.

3.1. Agarwood formation

A healthy tree of *Aquilaria* spp. will never generate resin compound. It has to be exposed to certain stimulants to stimulate plant defense mechanisms to act, hence, allowing the production of volatile compounds and aromatic secretion of resin. As demonstrated by Rahayu (2010), healthy wood transforms into agarwood after artificial induction. It is also found that sugar content in agarwood inoculant will help agarwood tree defense mechanism by restraining clump formation symptoms. *A. sinensis* converts substances found in the healthy tree into agarwood compound at eight months of incubation. Some available chemical substances in a healthy tree, such as dibutyl phthalate and phenol,2,2-methylenebis[6-11-dimethylethyl)-4-methyl] are decreased after fungi invasion (Cui et al., 2013). This discovery is supported by researchers (Chen et al., 2011) when different chemical compounds were identified in healthy trees, chemical-inoculated trees and natural agarwood. Substance conversion is initiated by signal molecules produced by fungi during infection. Thus, it stimulates gene expression linked to the synthesis of secondary metabolites and eventually their accumulation. However, compound concentration varies based on cellulose and lignin from the stem part (Novriyanti and Santosa, 2011). The first process of olio-resin production is taken in cells of the included phloem, rays, parenchyma para-trachea, and trachea. It is the tree's response to injury if its first line of defense, the formation of phloem callus tissue, is inhibited from forming over the wound (Gunn et al., 2004; Mulyaningsih, 2002). As shown in Figure 1, the agarwood compound will be produced after interaction with fungi (*Fusarium* sp.) and insects. Compounds found in agarwood were synthesized through lipogenase (LOX) and mevalonate pathway (MVA). The concerned pathway is the MVA pathway that converts acetyl Co-A to terpenoid, significantly essential to contributing to agarwood's unique fragrance.

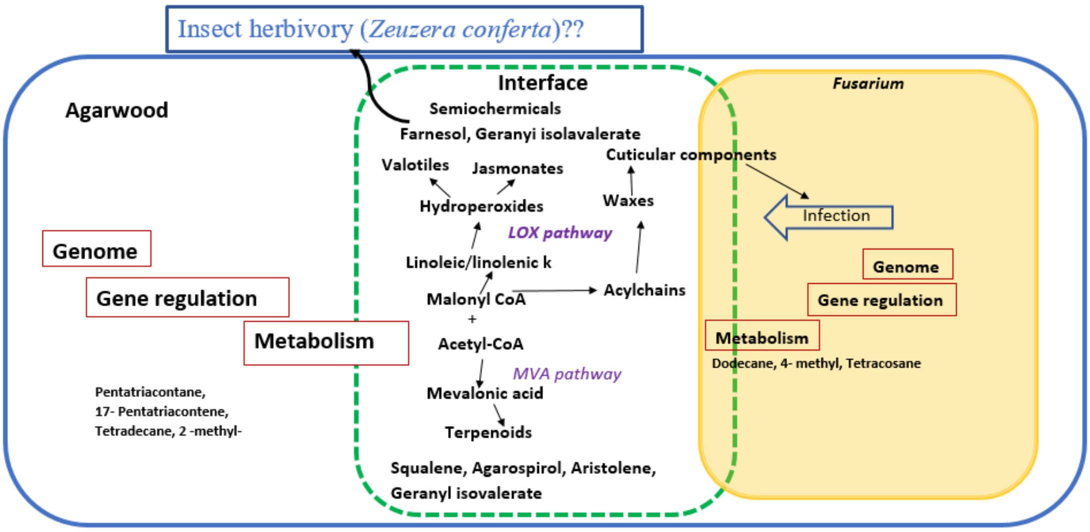

Figure 1. Interaction of fungi (*Fusarium* sp.) and insect with agarwood healthy wood lead to the production of biochemicals associated with agarwood (Pinney, 2011)

3.2. Plant defense mechanism

The plant defense mechanism will induce a secondary plant metabolite known as sesquiterpene. Sesquiterpene is one of the volatile plant compounds that can be antibacterial, antifungal or repellent to herbivores (Huang et al., 2012; Taniguchi et al., 2014). Sesquiterpenes are a terpene class consisting of three isoprene units and have the molecular formula $C_{15}H_{24}$. Sesquiterpenes may be acyclic or contain rings, including many unique combinations. Biochemical modifications such as oxidation or rearrangement produce the related sesquiterpenoids. Sesquiterpenes are found naturally in plants and insects (Zviely and Li, 2013). The formation of sesquiterpenes as a result of plant interruption was proven in the plant family solanacea (Santoso et al., 2011).

However, compound formation and accumulation of agarwood have critically relied on types of stimulants. Yet, specific responses mechanism before agarwood formation remain a mystery, where only seven percent of total agarwood from nature will be infected and produce agarwood (Akter et al., 2013). The healthy tree may respond differently to microbial infection that is eventually associated with agarwood formation through the level of disease and synthesizing chemical compounds (Santoso et al., 2011). Decomposed part of the trunk contributes to the scented properties of agarwood (Novriyanti and Santosa, 2011). When healthy trees are affected by fungi invasion and injury, plant cells begin to strengthen physical protection by cell thickening and lignification (Cui et al., 2013). As infection occurs at the injured part, tylosis may occur to cut off nutritional supply from being used by the pathogen (Akter et al., 2013). Tylosis is an abnormal growth of parenchyma cells in xylem vessels occurring in secondary heartwood when the tree is stressed or distraught. Apart from that, plant self-injury or self-death system is necessary to block fungal spread by stopping food supply and further infection areas with lignification (Bednarek, 2012; Cui et al., 2013). The plant defense system is part of surrounding management security (Bednarek, 2012). The alarm system will be triggered when the microbe-associated molecular patterns (MAMPs) receptor identifies a threat. Secondary metabolites are usually produced with antimicrobial and toxicity properties to stop the infection from spreading to other healthy plant cells. For instance, benzoxazinone glucosides (BXs) may be indicated by the disorganization of plant cell structure (Frey et al., 2009). Therefore, interruption in plant security may trigger the plant defense system to initiate the formation of secondary metabolites to rescue healthy cells. In agarwood, the secondary metabolite accumulation appears physically in the form of aromatic resin substance.

3.3. Fungi association in agarwood formation

In nature, agarwood formation comes from responses to fungal infection and physical wounding (Barden et al., 2000). Previous studies reported that more than one fungus species interact with *Aquilaria* sp. during agarwood formation and that one tree may have at least three fungi association (Mohamed et al., 2014; Nurbaya and Baharuddin, 2014; Rahayu, 2010; Santoso et al., 2011). For example, Santoso et al. (2011) thriven to isolate *F. sambunicum*, *F. tricinctum*, and *F. solani*, the most dominant species in agarwood samples from several locations in Indonesia. Involvement of *Fusarium* spp. is strongly proven by the growth of nine isolates on Potato Dextrose Agar (PDA) and Banana Leaf Agar (BLA) consisting of *F. solani*, *F. oxysporum*, *F. lateritium*, *F. comfactum*, and *Fusarium* spp. based on fungal morphology (Nurbaya and Baharuddin, 2014).

F. solani is a common pathogenic fungus and decomposer in Malaysia that exists in roots, stems, trees, and soil (Chehri et al., 2015; Shahnazi et al., 2012), and it also can be found in all agarwood samples. Characterization of *Fusarium* sp. was referred to Leslie and Summerell (2006), Hafizi et al. (2013), and Chehri et al. (2015). Generally, microconidia were mostly oval shape and non-septate, with occasional septate and elongated forms. Meanwhile, macroconidia will be produced at the inoculation point known as sporodochia. Macroconidia characteristics could be summarized as usually straight, some were slightly curved, 4-5 septa with mostly 5 septa, blunt, apical cell shape, and a barely notched basal cell.

Besides *Fusarium* species, new findings indicate that *Xylaria* spp. and *Lasiodiplodia* spp. could be involved in agarwood formation (Cui et al., 2013; Mohamed et al., 2010). *Mucor* sp. from the white wood sample, known as T3, turned out to be *Cunninghamella bainieiri* after a DNA sequence analysis based on the NCBI database. In contrast, unknown species from T8 and T9 resinous wood samples have been identified as *Lasiodiplodia theobromae* (Mohamed et al., 2010). Identification of *L. theobromae* was based on colony and conidia, which takes about four weeks to produce (Norhayati et al.,2016). *L. theobromae* found in Peninsular Malaysia was determined to be pathogenic against *Jatropha curcas*, *Acacia mangium*, and *A. crassicarpa* species (Sulaiman et al., 2012; Tarigan et al., 2011).

Other fungi found in the same research were *Curvularia* sp. and *Trichoderma* sp. All fungi identification was done based on a molecular approach without fungi plate culture. However, different fungi species were also discovered using molecular, and fungi culture approaches. Agar-plating technique revealed *Trichoderma* sp., *Alternaria* sp., *Fusarium* sp., *Curvularia* sp., *Cladosporium* sp., and *Phaeoacremonium* sp. from matured agarwood of *A. malacensis* in India (Premalatha and Kalra, 2013). These fungi were confirmed physically and genetically. In addition, although fungi *Preussia* sp. and *Phaeoacremium africana* could not be physically identified in plate culture, they were discovered after molecular analysis.

The interaction between agarwood and fungi leaves was found based on the biological activity changes in agarwood trees. Enzymes like cellulose, pectinase, peroxidase, and polyphenol oxidase showed higher activity in *A. malaccensis* infected with *Chaetomiun globosum* and *F. oxysporum* (Tamuli et al., 2011). Meanwhile, *Aspergillus* spp. isolates AR13 affect higher laccase, cellulase and ligninolytic enzyme in agarwood tree while breaking down tree carbohydrate in comparison with *Penicilium* sp., *Fusarium* sp., *Chaetomium* sp., and *Lasiodiplodia* sp. (Sangareswari et al., 2016). *Aspergillus* genus isolated from *A. sinensis* has been recorded in China (Cui et al., 2013) and in *A. malaccensis* in India (Premalatha and Kalra, 2013). In Peninsular Malaysia, *Aspergillus* spp. was widely found infecting food and soil and decomposed rubber tree and oil palm trees (Lee, 2012). Little has been discussed if *Aspergillus* spp. may exist as endophytes in agarwood trees. *Aspergillus* spp was reported as an endophyte in the plant *Taxus mairei*, (Zhang et al., 2008), soybean plant (Khan et al., 2011), and *Melia azedarach* (Li et al., 2012). Known as common soil-born fungi, *Aspergillus* spp. may interact with plants as a pathogen and endophyte.

The effect of fungi roles in contributing to the formation of the unique aroma of agarwood has become clearer in recent research. Accumulation of agarwood compounds infected with *Fusarium* showed the most variation in chemical compound types (44 compounds) compared to uninjured control and injured control of juvenile agarwood trees in Assam after three months-induction (Sen et al., 2017). Among 44 compounds, 11 (eleven) compounds were discovered in agarwood samples either as oil or chip wood which were different from previous research.

3.4. Artificial agarwood formation in plantation

Previous research discovered a fungal association with natural agarwood. Thus, the initiative to produce artificial agarwood started with the selection of fungi. More than one single species was found from the fungal screening of one agarwood sample (Cui et al., 2013; Mohamed et al., 2010; Premalatha and Kalra, 2013). Hence, resin accumulation in agarwood may be induced by the combination of fungal species action. Despite the findings, incorrect fungi choice and the combination will only bring perish to plant and agarwood quality. Inappropriate fungal induction may disrupt plant cells and degrade resin that has formed and left rotted (Putri et al., 2017). Although some fungal inoculant may affect negatively, effective fungal inoculant also shows physical changes to the part of the agarwood tree other than the stem. According to Rahayu (2010), in artificial inoculation using potential fungi species, chlorosis symptoms appear on leaves on the first and second branches closest to injected holes after a month of injection. When the frequency of inoculants increases, the symptom will be diverse in other parts of the plant. The second month after artificial inoculation, injection holes start to darken and their intensity increase.

The fungal effect varies depending on the duration of infection. It is claimed that the longer the infection occurs, the intensity of colour and disease area will increase, respectively. The area of infection will turn brownish or darker colour. Darker colour with the larger area of the infected area from the inoculated point was observed on six months of induced agarwood compared to three months of induced agarwood (Mohamed et al., 2014). The dark colour on the wood indicated agarwood formation where the resin was secreted during interaction with fungal inoculation. The intensity of colour suggested an abundance of various types of aromatic compounds. As in *A. sinensis*, it takes at least eight months of fungal inoculation before it can be transformed into an agarwood compound (Cui et al., 2013). While resin accumulation intensifies, the fungal community is decreasing slowly. Reviewing the agarwood formation pattern after inoculating with *F. solani*, *Cunninghamella bainieri*, and *L. theobromae*, the population of *F. solani* and *C. bainieri* declined except for *L. theobromae* as quantified using qPCR (Mohamed et al., 2014). The findings may be caused by the properties of resin in agarwood and the plant's action to defense its healthy cells. Mature agarwood has aromatic compounds such as anisylacetone, benzaldehyde, benzylacetone and some sesquiterpene groups; these compounds contribute to fragrance properties, and they also proved to be anti-microbial (Chen et al., 2011; Hendra et al., 2016).

4. Fungi as agarwood inoculant

Artificial agarwood is produced by injecting agarwood inoculant into the matured agarwood tree. Inoculant is believed to contain agarwood inducer factors, including fungi. According to Mohamed et al. (2010) and Rahayu (2010), inoculation using a combination of *Acremonium* spp. and *Fusarium* spp. is more reliable to induce the production of aromatic compound accumulation than single species. Better wood discolouration, length, the width of the infected area, and fragrance was determined with *Acremonium* spp. inoculation followed by *Fusarium* spp. inoculation after one week. Unfortunately, no agarwood chemical analysis was done to verify its chemical compound content. *Fusarium* spp. is the most favorable fungal candidate to be included in agarwood inoculant. However, the region of origin may differentiate the potential of *Fusarium* spp. as the best inducer. It was found that *Fusarium* spp. originating from Tamiang Layang, Indonesia, has the highest confirmed composition of agarwood. Meanwhile, *Fusarium* spp. from Maluku belongs to the highest total concentration of aromatic compounds found in artificial agarwood of *A. microcarpa* (Novriyanti and Santosa, 2011). The discovery was revisited when three *Fusarium* spp. as *F. solani*, *F. sambunicum*, and *F. tricintum* isolated from agarwood in Gorontolo area, Indonesia, proved to be the most effective agarwood inducer in *A. malaccensis* and *A. microcarpa* (Santoso et al., 2011). The fungi can induce agarwood within two to six months after inoculation. Inoculation of *A. sinensis* in China using various fungi such as *Pestalotiopsis* sp., *Xylaria* sp., *Fusarium* sp., *Trichoderma* sp, *Colletotrichum gloeosporioides*, *Xylaria* sp., *Chaetomium* sp., *Penicillium* sp., and *B. rhodina* and *B. rhodina* combined with formic acid induction produced high yield and high quality of agarwood after seven to 12 months (Tian et al., 2013).

Recent agarwood inoculation research finds that fungal inoculant is the best to induce sestquiterpene production. Inoculant composed of fungi *Phialophora* sp. and *Fusarium* spp. successfully generated 42.2 % sesquiterpene content, the highest value compared to control and chemically induced agarwood of *A. crassna*. The result was derived from two years inoculation period. Apart from fungi, some agarwood producer applies salt and chemical such as methyl jasmonate and hydrogen peroxide to agarwood tree to induce resinous wood (Kenmotsu et al., 2011; Zhang et al., 2014). Some non-biological inducers may have induced agarwood, while others tend to kill the tree.

5. Agarwood quality

In the agarwood market, there are different prices of agarwood based on its quality. The traditional measurement of agarwood quality is reliable among agarwood traders since no standard agarwood quality has been established until now. However, agarwood price classification is not rigid and subjective according to individual or culture. Meanwhile, current technology application is more rigid and accurate where compound detection and amount are specific, and the result is reproducible.

5.1. Agarwood chemical compounds

Fragrance in agarwood comes from volatile compounds in the terpene group. Terpenoid metabolism may cause the metabolic pathway past sesquiterpenoid production when harvested (Rahayu, 2010). Natural agarwood gets higher demand in the international market because natural agarwood gives a softer, unique scent than artificially inoculated agarwood. The chemical constituent is made differently based on artificial induction, natural induction and undisturbed healthy tree. Chen et al. (2011) found that a healthy tree has the highest total volatile at 95.68%, followed by natural agarwood at 92.16% and chemical induction at 90.01 %. However, as defined by aromatic wood, chemical induction agarwood and natural agarwood comprises sesquiterpenes and aromatics of 80.00% and 89.01% compared with a healthy tree with a significant fatty acid and alkanes. Some terpene sub-groups such as sesquiterpenes and aromatics found in chemical induction and natural agarwood are benzylacetone, guaia-1(10),11-dien-9-one, guaiol, hinesol, α-copaen-11-ol, baimuxinal, vanillin, α-humulene, α-agarofuran, elemol, agarospirol, aromadendrene oxide, and baimuxinal. Natural agarwood has the least composition of alkanes and fatty acids, which indicates the superior quality of its agarwood.

5.2. Production of terpenes

Any stress of threat to plant biological activity may induce the production of secondary metabolites, which allows the plant to adapt and survive. For instance, a rubber tree will produce latex when its stem is cut open, which means recovery. According to Angelova et al. (2006), the substance is released as an act of defense in the plant. Secretion of phytoalexin, latex, and terpenoid is proof of plant self-healing, counterattack to pathogens and self-protection against environmental pressure (Frost et al., 2008; Maffei, 2010). Plant are able to release volatile compounds as a mean to communicate to adjacent plants, to attract a predator to get on plant biological threats or as an act of defense (Degenhardt, 2009).

Terpene is one example of a volatile compound and plant secondary metabolite with more than 30.000 groups (Parveen et al., 2014). It is biosynthesized through the mevalonate pathway and released by abiotic factors, especially from temperature fluctuation by *Citrus* (Wang et al., 2012). Terpene production happens in cytosol and plastid, where cytosol operates the MVA pathway while plastid has 1-deoxy-*d*-xylulose 5-phosphate (DXP) pathway, as shown in Figure 2. Unlike abiotic factors, biotic factors such as microbes, fungi, and herbivores get in physical contact with targeted plant organs and can also stimulate terpene biosynthesis. As in *A. malaccensis*, any injuries and pathogen contact will stimulate the production of resin consisting of terpene. These have also been demonstrated by the production of azadirachtin-A, a type of tetranortriterpenoid, during infection of *Agrobacterium rhizogenes* with *Azadiracta indica* (Satdive et al., 2007).

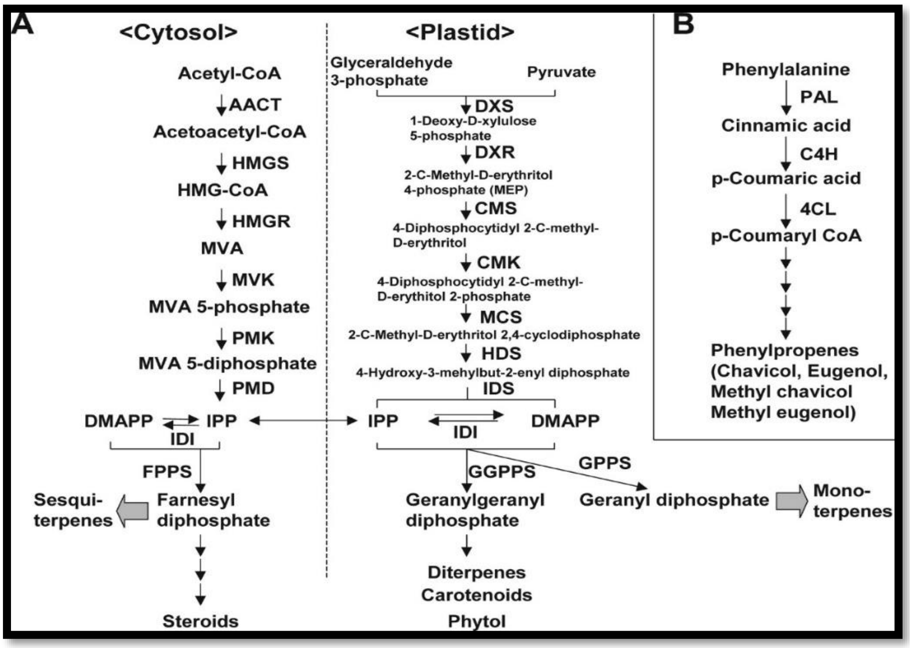

Figure 2. Terpene production happens in cytosol and plastid where cytosol operates MVA pathway while plastid has 1-deoxy-*d*-xylulose 5-phosphate (DXP) pathway.
MVA generally produces sesquiterpene from farnesyl diphosphate and gerayl diphosphate in DXP pathway.

6. Agarwood quality determination

Agarwood quality determination based on the traditional method is more associated with human scent and perception like aroma and country of origin. Agarwood physical properties such as density, colour, and scent are also applied in the agarwood grading system.

6.1. Human scent and perception

Individual enjoyment of different kinds of smell for leisure is different; some are already bonded with cultural practice. Agarwood may emit a sweet, woody and spicy aroma. In the agarwood direct selling market, the scent was the primary consideration for preference (Antonopoulou et al., 2010). Arabic people prefer woody and spicy aromas, while Chinese and Japanese inclined to choose sweet and fruity smells (Liu et al., 2017). These aromas may be significant to their country of origin resin content of the agarwood and *Aquilaria* species. However, agarwood with low quality will release an irritating burning aroma to the eyes and nose when burnt. A pleasant smell will only come from high-quality agarwood and the scent may remain in a room for a longer period depending on its resin and agarwood size (Antonopoulou et al., 2010; Liu et al., 2017). After all, agarwood quality based on human perception is unreliable as an indicator determining agarwood price in the general world market.

6.2. Agarwood physical properties

Resin content in agarwood may differentiate high-quality agarwood from low-quality agarwood based on colour density and sinkage.

6.2.1. Agarwood color

Resin accumulation after the agarwood tree is infected may affect the colour density of agarwood: the darker agarwood, the higher quality of agarwood (Barden et al., 2000). In the UAE agarwood market, sellers purposely polished agarwood to make it look darker than the original colour to attract buyers (Antonopoulou et al., 2010). Another trick was that sellers acquired immature agarwood and buried it in the soil for a certain time to make it look darker after prolonged decomposition (Heuveling van Beek and Phillips, 1999). Therefore, agarwood color was a disputable judgement for agarwood quality. Nevertheless, Malaysia agarwood still applies agarwood quality based on agarwood darkness named 'ABC' grading system. Grade A agarwood called 'kalambak' is dark brownish wood, Grade B is healthy wood with dense strips of brownish resin, while Grade C is a lesser brownish resin strip compared to Grade B (Liu et al., 2017).

6.2.2. Agarwood sinkage

Higher quality agarwood is considered to have entire sinkage property as it contains abundant resin. This method is to put agarwood in water and measure its position, either sinkage, half sinkage or floating. Sinkage agarwood indicates high-quality agarwood, half-sinkage to become medium-quality agarwood and finally, floating agarwood is considered low-quality agarwood (Liu et al., 2017). The sinkage justification was an inaccurate measurement, as not all high-quality agarwood will sink. The sinkage phenomenon is associated with wood density which accounts for types of *Aquilaria* spp. For instance, a lower grade of *A. malaccensis* can sink more profoundly than the high quality of *A. sinensis* since *A. malaccensis* has a compact texture and denser wood than *A. sinensis* (Liu et al., 2017).

6.2.3. Chemical analysis of agarwood

The quality of agarwood can be analyzed from agarwood oil, agarwood smoke or agarwood volatile compound. Compounds from chip wood samples were extracted using the Solid Phase Microextraction (SPME) method. This method allowed samples free from any alteration or disturbance by solvent where all volatile compounds evaporate directly from agarwood and adhere to SPME fiber. The method introduced by Arthur and Pawliszyn (1990) was known to be a rapid, sensitive and solvent-free method. Moreover, the SPME method is fast and straightforward in analyzing agarwood quality compared to any other extraction SPME method that requires fiber to trap volatile compounds (Meng et al., 2011; Pripdeevech et al., 2011). According to a study of volatile compounds from virgin coconut oil, divinylbenzene-carboxy polydimethylsiloxane (DVB-CAR-PDMS) was found to be the most suitable due to its high sensitivity to exposed standard combination with a relative standard deviation that is less than 10% of concentration (1.0 µg/g) compared to carboxen polydimethylsiloxane (CAR-PDMS), polydimethylsiloxane-divinylbenzene (PDMS-DVB) and polydimethylsiloxane (PDMS) coating fibers (Vichi et al., 2003). In addition, DVB-CAR-PDMS was firmly proven to be efficiently applied with a broader range of sample concentrations up to 5 µg/g and gave consistent results (Vichi et al., 2003).

GC-MS is a reliable instrument to detect compound composition in volatile substances based on compound mass. Previously, aromatic samples were qualified based on odour sensed by a human. However, it is subjective to rely on. On the other hand, GC-MS measures compound compositions based on gas chromatography and mass spectrometry. Gas chromatography uses a capillary column, which may vary with length, diameter, film thickening, and phase property. The molecules will be separated in the capillary column during GC-MS operation based on their molecular mass. Therefore, each unique compound will reach the end of the capillary column at different times due to its chemical properties. Compounds that move out of the capillary column will be ionized and detected based on the mass-to-charge ratio with a mass spectrometer (Hübschmann, 2015). GC-MS has been widely used to identify compounds in essential oil. GC-FID and GC-MS analysis helped discover of fundamental oil sesquiterpene group (Maggi et al., 2010). Previously, GC-MS application identified the main compounds of *Thymus corosus* Heuff or thyme named cryophyllene-oxide, camphene, β-bourbonene, eudesmol, and α-pinene (Pavel et al., 2009). As essential oil consists of monoterpene and sesquiterpene, GC-MS successfully differentiated *Tangetes minuta* L. or chinchilla elemental oil composition among different origins (Chamorro et al., 2012). These repeated operations of GC-MS also lead to agarwood composition analysis from other species, locations and maturity (Chen et al., 2011; Tsan and Mohamed, 2014). While GC-MS alone may not be robust data, GC-MS and GC-FID combination will give rigid data. GC-FID analysis is based on a flame ionization detector and is practically applied to organic compounds (Holm, 1999). GC-FID reflects unknown compounds according to their Kovats Index, calculated with known carbon standard retention time when GC-MS is limited with the available database. A combination of GC-MS and GC-FID has been practised with essential oil composition of *Coriandrum sativum* L, saffron and beer volatile analysis (Anastasaki et al., 2009; Charry-Parra et al., 2011; Msaada et al., 2007).

7. Commercial value of agarwood

High demand in the perfumery industry has led to illegal trade and threats to the agarwood tree population in its natural habitat. Natural agarwood reaches international prices from USD 2.000 to USD 16.000 per kilogram (PERsOOn, 2007). Agarwood planters generate luxurious income for their consistent high price. Agarwood is being exported in the form of chips, powder and oil. Price is dependent on resin content and purity of agarwood. Agarwood chips may be sold from USD 29.0 to USD 8888.0 per kilogram. High-quality agarwood starts at USD 1.000/kg (PERsOOn, 2007). For every initial investment of USD 32.000, agarwood traders can gain a return of USD 104.000. Hence, the trader made up a 29% Internal Rate of Return within seven years of trading (Akter et al., 2013). Meanwhile, agarwood essential oil costs between USD 50.000 to USD 80.000 per litre, requiring extraction of 100 to 150 kg of agarwood (PERsOOn, 2007).

International export value is estimated at USD 14 million, which is 43% of the total retail value. Laos produces agarwood supply to the world worth USD 33 million, where the majority is contributed by a foreign harvester who exports about 18 tonnes of agarwood to Vietnam (Jensen, 2009). If the East-Asian market is considered, estimates of overall retail value from Laos would be higher, USD 40 to 45 million. In 2004, international trade in agarwood was assumed to reach USD 2.3 billion (Jensen, 2009). Mai dii, a common name for high-grade agarwood in Laos, costs more than THB 500/kg for raw agarwood. Medium grade, known as mai khilai, goes for extraction of top-quality essential oil and is marketed at THB 80 to 500 per kg (Jensen and Meilby, 2010). These harvesters collect profit when they can better network with big agarwood industry players. They sell agarwood at higher prices when agarwood industry players gain the advantage of value-added agarwood products and earn more than harvesters (Jensen and Meilby, 2010).

As a consequence of this scenario, agarwood industry players are torn between limited high-quality resources and high prices for business sustainability. Another alternative to agarwood is derived from a chemical process despite the highly preferred natural agarwood (Akter et al., 2013). Japan maintains its demand for the rare top quality natural agarwood (PERsOOn, 2007) and the United Arabic Emirates is included in using natural agarwood in religious rituals. Excellence in commercial markets and trade is the leading cause of *Aquilaria* sp. extinction.

Overharvesting, uncontrollable illegal logging and reserved forest invasion by local or neighbouring citizens bring *Aquilaria* sp. loss and habitat to endemic flora and fauna (Akter et al., 2013). *A. malaccensis* is in the list of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (Inskipp and Gillett, 2005). Therefore, rather than hunting agarwood in their natural habitat, conducive climate countries have initiated their agarwood plantation as in Bangladesh, Bhutan, India, Indonesia, Laos, Malaysia, Myanmar, Vietnam, Australia, and Thailand.

8. Application of agarwood

Nowadays, agarwood is in top demand in the perfumery industry. Nevertheless, the multipurpose of agarwood has made it a demanded forest source for decades. Incorporating agarwood aroma into human life and discovering traditional agarwood practices open a new field for researchers to explore the further discovery of this agarwood potential. Continuous agarwood practice in human lifestyle and lengthy application in health improvement bring agarwood to become a world brand, although its supply is depleting each year.

8.1. Agarwood in culture and ritual

Since ancient times when our ancestors accidentally found agarwood in the forest, they brought agarwood into usefulness and adapted it to the local culture. For the Malay race, it is widely used in the range of perfume, religious purposes, spiritual rituals, and decorative carvings (Chua, 2008; Saikia and Khan, 2012). Even today, some agarwood field practitioners made low-quality agarwood into attractive shapes and bracelets for sale. For Muslims, non-alcoholic perfume is preferred during prayer because agarwood-originated perfume has become popular among Arabic people. Besides, the simplest way to enjoy agarwood fragrance is by burning, happily applied to the luxury incense-making industry. Agarwood incense would not contain total agarwood composition due to its limited supply and high price. Nonetheless, agarwood incense is used in the religious ritual activities of Muslims, Buddhists and Hindus (Akter et al., 2013).

8.2. Agarwood in health and medicinal purpose

Apart from being an aromatherapy material, in medicinal prospect, agarwood can be used as a carminative, stimulant for heart palpitation, tonic during pregnancy, remedy during the post-natal recovery period, and cure for the disease of the female genital part. The whitebark of agarwood is believed to heal jaundice and body pain (Ali et al., 2016). Moreover, agarwood helps to relieve body pain, warm abdomen, relieve asthma, treat coughs, and acroparalysis, and acts as an anti-histamine, analgesic, and anti-inflammatory (Bhuiyan et al., 2009; Chitre et al., 2007). In Chinese Traditional Medicine, it is helpful as a sedative to relieve gastric problems, relieve rheumatism and high fever (Liu et al., 2017). Various compounds in agarwood can fight cancer cells but the information is limited (Dahham et al., 2014). Sesquiterpene compounds in agarwood pose antimicrobial properties and they are effective, especially to gram-positive bacteria (Chen et al., 2011).

8.3. Agarwood as perfume

Practice commonly among Arabic culture and agarwood lovers has spread worldwide as a component in fragrant commercial brands. In Yemen culture, agarwood aromatic is indulged in several ways (Jung, 2011). The smoking odour of raw agarwood chip wood is applied during prayer, special occasions and daily home refresher. Usually, dense, resinous agarwood turns into incense compared to low-quality agarwood. Lower-grade wood will be distilled to extract pure agarwood oil. Some people, mainly Arabic, enjoy the original woody, balsamic scent of agarwood oil. However, agarwood oil may undergo adulteration to satisfy a wider range of people's preferences in fragrance (Jung, 2013). It can be blended with other fragrances as base notes to lengthen the fragrance effect. Another way to add value to lower-grade agarwood is to immerse low-grade chip wood in agarwood oil before being sold as incense, prayer bead, sculpture and home decoration (Jung, 2011). Agarwood oil from different species and regions will emit different scents due to their difference in the top note, such as fruity, animal and leathery odour (Zviely and Li, 2013). Notes depend on the volatility of compounds in particular fragrances. Top notes have the highest volatility, followed by medium and base notes. In the agarwood case, regardless of species and region, the oil will definitely emit a woody smell after seven to eight hours of skin application. These woody smells may be contributed by compounds such as eremophila-9,11-dien-8-one, (-)–guaia-1(10), 11-dien-15-al, and jinkohol II (Zviely and Li, 2013).

9. Conclusions

Plant compounds derived from agarwood have long been utilized as traditional remedies in Southeast Asian societies and Chinese, Tibetan, Unani, and Ayurvedic medicine. They have sedative properties and are used to treat arthritis, asthma, and diarrhoea. Natural agarwood induction periods were unknown but considered acceptable for harvesting and marketing by Kedaik agarwood practitioners. Agarospirol, β-selinene and 10-epi-γ-eudesmol were detected in artificial and high-quality natural agarwood, where agarospirol existed in higher abundance in artificial agarwood. Many pharmacological studies on crude extracts have been conducted. These extracts exhibit anti-allergic, anti-inflammatory, anti-diabetic, anti-cancer, antioxidant, anti-ischemic, antimicrobial, hepatoprotective, laxative, and mosquitocidal properties and central nervous system effects. Future research should focus on bioassay-guided isolation of bioactive molecules with adequate chemical characterization, as well as insights into the underlying processes for drug development. By tying agarwood ethnopharmacology to reported pharmacological qualities, anti-inflammatory capabilities may be the future study area, as inflammation underpins many disease states.

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Conflict of interest

The authors have no relevant financial or non-financial interests to disclose.

Statement of ethics

In this study, no method requiring the permission of the “Ethics Committee” was used.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

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Supplementary File

None.

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