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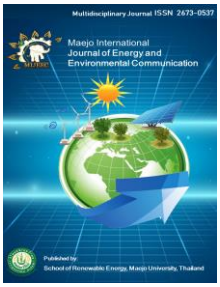

ARTICLE

Gas chromatography analysis of the microwave-aided extracted agarwood oil from physically induced *Aquilaria malaccensis* trees in Northern Thailand

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ABSTRACT

Agarwood (*Aquilaria malaccensis*) oil is one of the essential bioactive materials in the scent/ perfume industry for the middle east and gulf countries. The built-in functionality and availability of bioactive components of oil make agarwood more precious. In this study, the physically induced infected agarwood tree chops were extracted by microwave assisted Soxhlet extraction. Moreover, the extracted oil samples were analyzed against control not infected trees using Gas chromatography-mass spectroscopy (GC-MS) by GCMS (Agilent GC 6890zN MS 5973). The microwave assisted Soxhlet extraction revealed a higher amount of oil extraction. In the physically induced samples, 24 signal peaks were recorded employing GC-MS. The most prominent peak area recorded was -5-hydroxy-3-methyl-1-indanone detected at minute 41.89 with a peak area of 17.64%, followed by -methyl 4,4,7-trimethyl 4.7-dihydro indan -6- carboxylate at 23.17 min, occupying an area of 15.54%; followed by 3-carene recorded at 46.61 minutes with an area. According to this research, the microwave-assisted Soxhlet extraction method outperformed and can be utilized in the agarwood industry for enhanced oil production.

1. Introduction

Aquilaria malaccensis is a tropical tree that produces agarwood in its trunk after being injured or attacked by pathogens or insects (Huda et al., 2009; Ramli et al., 2021a, b). The production of naturally synthesized agarwood takes up to 30 years, limiting its availability (Faizal et al., 2017). The plant defence 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).

Sesquiterpenes are found naturally in plants and insects (Zviely and Li, 2013). The essential agarwood oil can be used in cosmetics, personal care, incense, and therapeutics. Agarwood remains an integral part of spiritual and secular life in many cultures where it has been used traditionally. In many Middle Eastern societies, agarwood is used as a symbol of wealth and

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status, particularly during religious rites and social occasions, to infuse personal and domestic items with its distinct fragrance (Antonopoulou et al., 2010). The high prices paid for agarwood products have stimulated activity in the tree's cultivation (Quang and Huu, 2011) and renewed interest in artificial stimulation of agarwood formation (Quang and Huu, 2011, Thuy et al., 2019, Liu et al., 2013). These initiatives are thought to potentially create alternative agarwood sources and increase the market availability of agarwood products (Nakashima et al., 2005).

The technology creates agarwood by creating an artificial wound in the stem and inserting a tube into the wound to aid in aeration. To stimulate agarwood formation, various chemicals such as brown sugar, formic acid, hydrogen peroxide, methyl jasmonate, salicylic acid, sodium chloride, and soybean oil have been used (Chen et al., 2011, Ito et al., 2005, Wei et al., 2012, Zhang et al., 2014). These chemicals are introduced into the plant's xylem. Trees in varying concentrations cause damage to the entire plant due to being distributed throughout the plant via xylem transportation (Chhipa et al., 2017).

GC-MS analysis of MeJA-treated shoots revealed the presence of several agarwood compounds, including sesquiterpenes and chromone derivatives (Faizal et al., 2021). A high concentration of Methyl jasmonate caused necrotic shoot tissue, whereas crude extracts did not affect shoot growth. The ether extract oil of white wood had a 1.80% ether content, screw-injected wood had a 20.49% ether content, and insect-infested wood had an 11.078% ether content. The phenolic contents of white wood, screw-injected wood, and insect-infested wood were 2.55 mg/g, 3.6 mg/g, and 2.97 mg/g, respectively (Hoque et al., 2019). This study aims to compare the chemical composition of crude extracts from *Aquilaria malaccensis* infected woods to healthy wood for commercial agarwood production.

2. Material and Methods

2.1 Samples collection

The study area was located in the highland's region of Doi Saket, a district in the eastern part of Chiang Mai province in northern Thailand. Raw material samples were collected from physically induced tree chips from the Doi Saket agarwood farm (Figure 1). In contrast, the Control sample was taken from the fresh tree without any injected inoculants. After being harvested, small wood chips were crushed into a fine powder (Figure 2).

Figure 1 Doi Saket farm: Natural trees and physically induced agarwood trees

Figure 2 Infected agarwood chips (left) and fine grounded powder of agarwood (right)

2.2 Extraction of bioactive compounds

Extraction of bioactive compounds was attained by microwave assisted Soxhlet extraction (ETHOS™ X Microwave Extractor, Italy). The process was initiated by soaking 200g of agarwood powder in 2L of distilled water. On the first day, the extraction was done by setting the microwave power at 800W for 6 hrs. The process started with vaporization and condensation at about 1 hour and continued until 6 hours. On the 2nd day, set the power of the microwave at 1000 watts for 6 hrs. The results of the extraction experiment yielded approximately 0.1 -0.2 ml. The extracted oil sample was collected and stored at room temperature for GC-MS analysis.

2.3 Gas Chromatography-Mass Spectroscopy (GC-MS) analysis

The microwave-assisted extracted samples were analyzed using GCMS (Agilent GC 6890zN MS 5973) inert, HP-5 (30 m x 0.25 mm x 0.25 μm). The specification of the method was as follows: the oven temperature was ramped from 70 ℃ (hold for 5 min) to 100 ℃ at a rate of 50 ℃/min to 200 ℃ at a rate of 1℃/min to 250 ℃ at a rate of 5 ℃/min (hold 5min). The injection and detector temperatures were 250 ℃ and 280 ℃, respectively. The flow rate of Helium carrier gas was set at 1 ml/min. In GC-MS analysis, stored samples were prepared for both control and physically induced samples after extraction.

3. Results and Discussion

3.1 Sample preparation

Microwave-assisted Soxhlet extraction (ETHOS™ X Microwave Extractor, Italy) was employed to extract bioactive compounds. The extraction was done on the first day by setting the lower microwave power (800W/ 6 hours). The process began with vaporization and condensation for 1 hour and continued for 6 hours, resulting in a brown substance on top of the solvent, which resembles resin or oil. The maximum temperature was raised to 91 ℃. The treated solution has a darker brown colour than the untreated solution. On the second day, the microwave power was increased (1000 watts/ 6 hours). The improved power results in faster steaming and condensation at about 30 minutes (Figure 3).

The extraction method affects the yield and chemical compounds of gaharu oil. The extraction yields and percentages of five specific chemical compounds, namely 3-phenyl-2-butanone, -guaiene, -agarofuran, 10-epi-eudesmol, and agarospirol, were investigated using hydro distillation, Soxhlet, and accelerated solvent extraction (ASE). ASE was the best method for obtaining a large amount of gaharu oil containing the five chemical

compounds with the highest total compound percentage (Sulaiman et al., 2015).

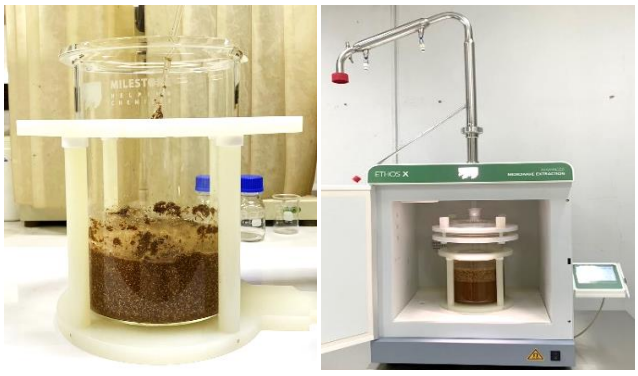

Figure 3 The microwave-assisted extraction in ETHOS™ X Microwave Extractor

3.2 Screening of the GCMS

The samples prepared with different pre-treatment methods were analyzed using GCMS Agilent GC 6890zN MS 5973 inert, HP-5 (30 m x 0.25 mm x 0.25 µm) with the procedure stated in 2.3 with the split mode. The GC-MS results revealed non-infected (control), and physically induced infected agarwood samples were displayed in Figure 4.

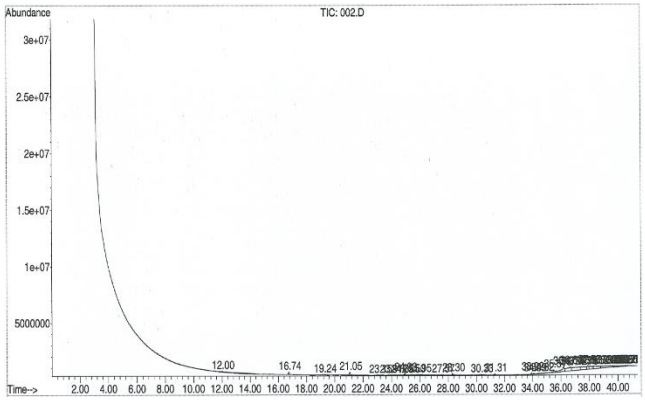

Figure 4 The GC-MS peaks of non-infected (control) agarwood samples

In the control sample (Figure 4), the GCMS peaks were recorded from 12.05 to 40.77 min, with a total of 34 signal peaks. 2-pyridinepropanoic acid was detected at the following time points: 37.27, 37.75 and 38.53 with areas of 13.34, respectively, 9.7 and 8.92%. The second largest area was (tetrahydroxycycloo-nepentadienone) tricarbonylion recorded with 10.19% area at 39.52 min. The following positions are -4-bromo-2,5-dimethoxyamphetamine, respectively, which were recorded at 9.74 and 9.69% at the time point from 36.17-36.64%. 3,6-dimethypiperazine -2,5-dione was detected with 6.45% after 38.03 min. Other compounds were detected between 12.05-40.77 min with an area not greater than 5%.

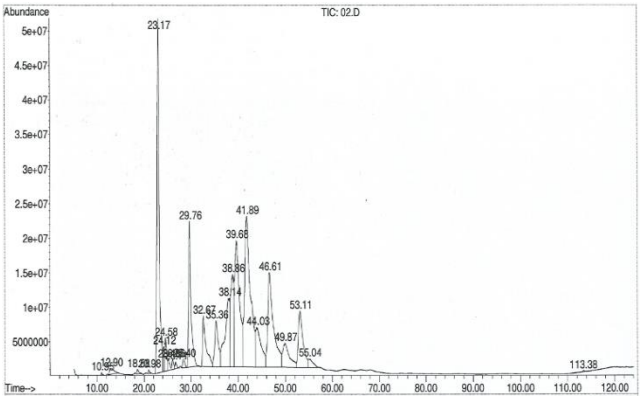

Figure 5 The GC-MS peaks of physically induced infected agarwood samples.

In physically induced samples (Figure 5), a total of 24 signal peaks were recorded. The first GCMS signal was detected after 10.59 with an area of 0.04 belonging to benzene and the final compound detected after 113.37 minutes, with an area of 0.01% be appropriate to the Bis(2-ethylhexyl) phthalate class of substances. The compounds were detected in high concentrations during the 20-50 min GCMS run. The largest peak area recorded was -5-hydroxy-3-methyl-1-indanone detected at minute 41.89 with peak area of 17.64%; followed by -methyl 4,4,7-trimethy 4.7-dihydro indan -6- carboxylate at 23.17 min, occupying an area of 15.54%; followed by 3-carene recorded at 46.61 minutes with an area of 10.71%. Other large-area signals recorded were (2Z,6E)-3.7-dimethyl -9-(1-methylethyl hylidenyl)-2.6-cyclodecadiene -1-one at 29.67 minutes, 6.74%, respectively; cyclohexane was detected at 6.39% at 38.15 min and 2(3H)-naphthalenone with 5.27% detected at 53.11 min. Other compounds were also detected between 10.59 and 113.37 min but in less than 5% area.

Gas chromatography-mass spectrometry (GC-MS) analysis of the inoculated samples revealed some important agarwood compounds such as tridecanoic acid, -santalol, and spathulenol, which were absent in both healthy controls and only wounded samples by injecting and inoculating cultivated *Aquilaria malaccensis* with four strains of *Fusarium solani* isolated from various locations in Indonesia (Faizal et al., 2017). Crude extracts from *Aquilaria malaccensis* infected woods were compared to healthy wood and commercial agarwood in terms of their chemical composition. The 2-(2-phenylethyl) chromone derivative, 4-phenyl-2-butanone, and other significant chemicals were detected in the agarwood extract of the young fungal-elicited tree but not in the healthy wood (1S,4S,7R) -1,4-dimethyl-7-(prop-1-en-2-yl)-1,2,3,4,5,6,7,8-octahydroazulene [guaiene], 1,1,4,7-tetramethyl-2,3,4,5,6,7,7a,7b-octahydro-1aH-cyclopropa[h] Azulen-4-ol (palustrol), 4-(4-methoxyphenyl) butan-2-one (anisylacetone). Along with agarospirol, alloaromadendre oxide (2), -elemol, -eudesmol, and guaiol, these were also discovered in agarwood of various grades and agarwood that was harvested from a mature wild tree (Tsan and Mohamed, 2014).

4. Conclusion

The extraction of agarwood essential oil needs further study because the extraction time is longer than one day. Microwave oven-assisted extractions can be an efficient method. Also, this method may lead to the loss of volatile compounds by soaking the

agarwood powder sample in water before it evaporates. It is necessary to improve or change the extraction method of agarwood oil to ensure efficiency, avoid loss of volatile compounds and solubility, and easily destroy or denatured compounds in the water solvent. At the same time, using a large number of samples in this extraction method is quite wasteful; it is necessary to improve the extraction method to save samples because agarwood is a plant of high commercial value. Through testing by GCMS, the concentration of detected compounds in the injected sample is higher than in the control sample. However, the detection time was much longer than the control. The substances detected in the experimental samples were not entirely like those in the control plants in quantity and type. Future attention and expanded work are required to gain many organic compounds with efficient extraction methods.

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