Research article

Effect of Microwaves, Ultrasound, Enzyme, and Lactic Bacteria on Extraction Efficiency of Bioactive Compounds from Agarwood Leaves (*Aquilaria* spp.)

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Abstract

Agarwood leaves (*Aquilaria* spp.) have been shown to benefit human health due to their bioactive compounds. However, studies on extraction methods for these beneficial compounds from agarwood leaves have been poorly reported. Therefore, the present study aimed to investigate the efficiency of extraction methods for such compounds. The studied methods included microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), Viscozyme L treatment, and fermentation by *Lactobacillus acidophilus* ATCC 4356. The total contents of polyphenols, polysaccharides, saponins, flavonoids, and antioxidant activity were evaluated. The results showed that bioactive compound extraction efficiency differed depending on the treatment methods. Viscozyme L treatment showed the most effective method, followed by MAE treatment and lactic fermentation, and UAE treatments showed the lowest result. The total contents of polyphenols, polysaccharides, saponins, flavonoids, and the antioxidant activity in the case of Viscozyme treatment were 51.81±2.65 mg GAE/g sample; 23.711±1.26 mg GE/g sample; 252.77±4.32 mg OAE/g sample; 7.90±0.72 mg QE/g sample; and 55.1±1.3%, respectively. The results also indicated that each extraction method has its advantages. MAE and UAE treatments caused overheating inside the medium, affecting

Keywords

Aquilaria spp.;
bioactive compounds;
lactic fermentation;
microwave-assisted extraction;
ultrasound-assisted extraction;
Viscozyme L

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the antioxidant activity compounds. In the case of the fermentation process, excessive prolonging of the fermentation time also affected the sensitive antioxidant activity compounds released from the plant cells. The lactic fermentation process required the most time to reach extraction efficiency. However, fermentation would provide bioactive compounds and probiotics for health benefits, showing a potential approach for food supplementation.

1. Introduction

Agarwood (*Aquilaria* spp.) is an evergreen tree that is widely known in China, Indonesia, India, Malaysia, and Vietnam. The tree grows to a height of up to 40 m, with a diameter of up to 60 cm, and has leaves of 5 to 9 cm in length, and of oblong-lanceolate shape [1]. People have cultivated the agarwood tree for centuries mainly because of the outstanding economic value of the agarwood resin. Products with fragrances from agarwood include essential oils, perfumes, shampoos, and fragrances. Benefits of frankincense from parts of the tree such as leaves and stems are diverse, and has long been used for psychophysiological, spiritual, therapeutic and medicinal purposes. Plant age is a significant factor in resin formation, as plants mainly produce resin of the best yield in terms of quality and quantity from 15 years of age and older [2]. However, it is not just the agarwood resin that has been shown to offer economic benefits. In previous studies, many important chemical components in agarwood leaves were identified, including 2-(2-Phenylethyl) chromones, triterpenes, glycosides, phenolics, steroids, terpenoids, xanthonoids, flavonoids, and nucleosides [3]. Moreover, it was noted that it might be possible to take economic advantage of agarwood leaves over the long period of wood maturation [4]. The potential pharmacological activities of agarwood compounds were reported for potent antioxidant effects, anti-diabetic, anti-inflammatory properties and so on [5-7]. Besides, extracts of agarwood leaves were found to have intestinal peristalsis activity in the small intestine [8]. These suggest that bioactive compounds from agarwood leaves have many health benefits, and extracting bioactive compounds is necessary to improve the efficiency of bioactive substances. However, a typical extraction process such as boiling or heating used in the dissolution process can cause degradation and oxidation of the valuable bioactive substances present in the leaves. Thus, researchers have turned to the development of less destructive and more efficient methods of extraction [9]. Previous studies have shown that the extraction process can be assisted by ultrasound, enzyme, fermentation, methanol extraction, and ethanol extraction, which can improve bioactive compound recovery efficiency from algae, plants, etc. [6, 10, 11].

A study by Habeebullah *et al*. [10] showed that the enzyme extraction process released compounds and increased the overall yield of bioactive compounds from brown algae. Similarly, lactic fermentation by *Lactobacillus* sp. was also known as one of the methods for efficiently extracting bioactive compounds from *Anoectochilus formosanus* Hayata [11]. These studies indicated that lactic fermentation and enzyme-assisted extraction could be a solution to extract bioactive compounds from agarwood leaves efficiently. However, previous studies focused on only one group of bioactive substances or used individual impact factors (such as microwave, ultrasonic) for the extraction method. The content of bioactive compounds from agarwood leaves varies greatly depending on geographical location, leaf age, harvest season, etc. Therefore, the efficacy of different extracting methods should be evaluated and compared on the same object. This study was conducted to compare and evaluate the effectiveness of microwave, ultrasonic, enzyme, and lactic fermentation interventions on the extraction of bioactive compounds from agarwood leaves. The evaluated parameters were total polyphenol content, total polysaccharide content, total saponin content, total flavonoid content, and antioxidant activity.

2. Materials and Methods

2.1 Plant material

Agarwood trees (*Aquilaria* spp.), located at 10°51'20.0" N 106°57′28.3E in the Southeast region of Vietnam, were grown until the age was 5-6 years old. Leaves (Figure 1) of the same color and size were washed, dried with absorbent paper, and then shredded in a blender (HR2118/01, Philips, Netherlands). Four grams (±0.01) of sample were added to 100 mL of distilled water (pH 5.8-6.2) and analyzed in subsequent steps. Sample mixtures without any treatment were used as control samples.

Figure 1. *Aquilaria* spp. leaves

2.2 Microorganisms and culture

Lactobacillus acidophilus ATCC 4356 was obtained from the Faculty of Food Science and Technology strain collection, Ho Chi Minh City University of Industry and Trade. *Lactobacillus acidophilus* ATCC 4356 was multiplied on Man Rogosa Sharpe (MRS) at 37°C. After 24 h of culturing, biomass was collected and kept for use in the next step.

2.3 Extraction method

2.3.1 Microwave-assisted extraction (MAE)

The extraction was performed on a microwave oven (EMM2308X, Electrolux, Sweden) at powers of 300W, 450W, 650W, and 800W, for 1, 3, 5, and 7 min, respectively (10 s on the pulse and 20 s off pulse). Then, each mixture sample was centrifuged (Z206A, Hermle, Germany) at 3720 x *g* for 10 min. The extracts were then examined for bioactive compounds.

2.3.2 Ultrasound-assisted extraction (UAE)

The experiment was performed on an ultrasound machine (Q500, Qsonica, USA) with a maximum power of 750W and a frequency of 20 kHz. The agarwood leaf samples were subjected to ultrasound at 20%, 25%, 30%, 35%, and 40% of the machine's capacity with the time of 5, 10, 15, and 20 min (ultrasound for 10 s, rest for 10 s). Each mixture was then centrifuged at 3720 x *g* for 10 min. The extracts were then examined for bioactive compounds.

2.3.3 Treatment with Viscozyme L

The experiment was conducted by adding Viscozyme L (Novozymes A/S, Bagsvaered, Denmark) with a concentration of 1%, 1.5%, 2%, 2.5%, 3%, 3.5% (v/v) to each mixture sample and incubated for 1, 3, 6, 9, 12, and 24 h. After that, the mixture was centrifuged at 3720 x *g* for 10 min. The extracts were then examined for bioactive compounds.

2.3.4 Fermentation with Lactobacillus acidophilus

The experiment was conducted using the biomass of *Lactobacillus acidophilus*, which was determined by a spectrophotometer (based on a standard curve), and plate count on MRS agar to achieve a concentration of approximately 6, 7, and 8 log CFU/mL. The mixture sample with the addition of *L. acidophilus* was then fermented for 36 h at 37ºC and evaluated at 0, 2, 4, 8, 12, 16, 20, and 24 h of fermentation. After processing, each mix was checked for microbial density and centrifuged at 3720 x *g* for 10 min. The extracts were then examined for bioactive compounds.

2.4 Analytical method

2.4.1 Determination of total phenolic content

The total phenolic content was carried out following the method outlined by Vuong *et al*. [12] with certain adjustments. Specifically, 5 mL of Folin-Ciocalteu reagent was combined with 1 mL of the diluted sample, thoroughly mixed, and allowed to react for 5 min. Subsequently, 4 mL of sodium carbonate 7.5% (w/v) was added, and the mixture was incubated in darkness for 1 h. The absorbance was then measured at a wavelength of 765 nm. The results were expressed in terms of mg gallic acid equivalents (mg GAE/g sample).

2.4.2 Determination of total polysaccharide content

The total polysaccharide content was determined following the procedure outlined by Dong *et al*. [13], with some modifications. Initially, 1 mL of the sample was combined with 5 mL of 96% ethanol (v/v) and allowed to cool to 40°C for 24 h. The precipitate was collected, dissolved, and mixed with 10 mL of hot water at 70°C. Subsequently, 2 mL of this diluted solution was added to 8 mL of anthrone reagent in a concentrated sulfuric acid medium. After incubating at room temperature for 30 min, the absorbance was measured at a wavelength of 630 nm. The results were expressed in mg D-glucose (mg GE/g sample).

2.4.3 Determination of total flavonoid content

The total flavonoid content was determined following the method outlined by Zhishen *et al*. [14], with some modifications. Initially, 1 mL of the sample was mixed with 4 ml of distilled water, and 0.3 mL of sodium nitrite 5% (w/v) and thoroughly shaken. Next, this mixture was combined with 0.3 mL of aluminum chloride 10% (w/v) and allowed to react for 5 min. Subsequently, 2 mL of sodium hydroxide (1M) was added, followed by two dilutions with distilled water. The resulting solution was left at room temperature for 10 min, and the absorbance was measured at a wavelength of 510 nm. The results were expressed in mg quercetin equivalents (mg QE/g sample).

2.4.4 Determination of total saponin content

The total saponin content was determined following the method described by Chen *et al*. [15], with some modifications. Initially, 0.2 mL of the sample solution was combined with 0.2 mL of vanillin-acetic acid 5% (w/v). Subsequently, 1.2 mL of perchloric acid was added, and the mixture was incubated at 70°C for 20 min. Afterward, the solution was diluted to a final volume of 5 mL with ethyl acetate. The absorbance of the resulting mixture was measured at a wavelength of 550 nm. The results were expressed in mg oleanolic acid equivalents (mg OAE/g sample).

2.4.5 DPPH antioxidant activity

The DPPH antioxidant activity was conducted following the procedure outlined by Shimada *et al*. [16], with some adjustments. Initially, the extracts were diluted five times. Next, the polysaccharides were separated by incubating the extract mixture with ethanol (1:2, v/v) and centrifuging it at 3720 x *g* for 10 min. The solution was collected. Subsequently, 1 mL of the solution was combined with 5 mL of 0.1 µM DPPH in methanol. After allowing it to reach room temperature for 60 min, the absorbance was measured at a wavelength of 517 nm. The results were expressed as percentage inhibition.

2.4.6 Statistical analysis

The experiments were done in triplicate, and the results were expressed as the mean±standard deviation. Microsoft Office Excel 2019 and Sigmaplot 11.0 statistical software were employed for analyzing the results with 95% confidence ($p<0.05$) to compare the differences between the treatments using the Tukey and Duncan tests.

3. Results and Discussion

3.1 Effect of microwave-assisted extraction on bioactive compounds from agarwood leaves

The effects of microwaves on the total content of polyphenols, saponins, polysaccharides, and flavonoids, and on the DPPH antioxidant activity of *Aquilaria* leaves are shown in Figures 2 and 3. The results showed that microwave power and time had a significant effect ($p<0.05$) on the total contents of polyphenols, polysaccharides, saponins, flavonoids, and on DPPH antioxidant activity compared with the control. At the powers of 300W, 450W, and 650W, increasing the microwave time (from 1 min to 5 min) increased the total contents of polyphenols, polysaccharides, and the DPPH antioxidant activity, which then decreased when the microwave time was extended. The total polysaccharide content and DPPH antioxidant activity reached their highest values at 650W with

21.61±1.32 mg GE/g sample and 58.97±1.4%, respectively, after five min of treatment (Figure 3). The total saponin content increased with microwave time, but after 5 min, the content did not change significantly ($p< 0.05$). At 800W, the total contents of polyphenols, flavonoids, and saponins increased and reached their highest values when the microwave time was increased from 1 min to 3 min, with 59.29±1.68 mg GAE/g sample, 6.15±0.44 mg QE/g sample, 251.16±3.76 mg OAE/g sample, respectively. With further increase in microwave time, the contents decrease sharply (Figures 2 and 3).

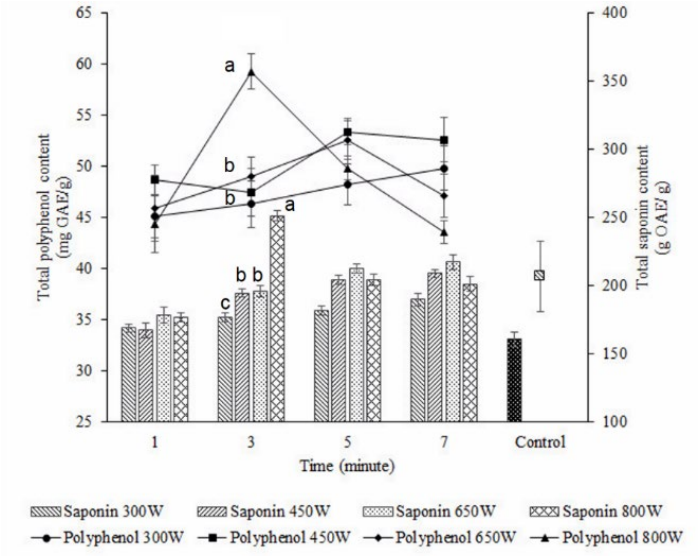

Figure 2. Effect of MAE on the total content of polyphenol and saponin

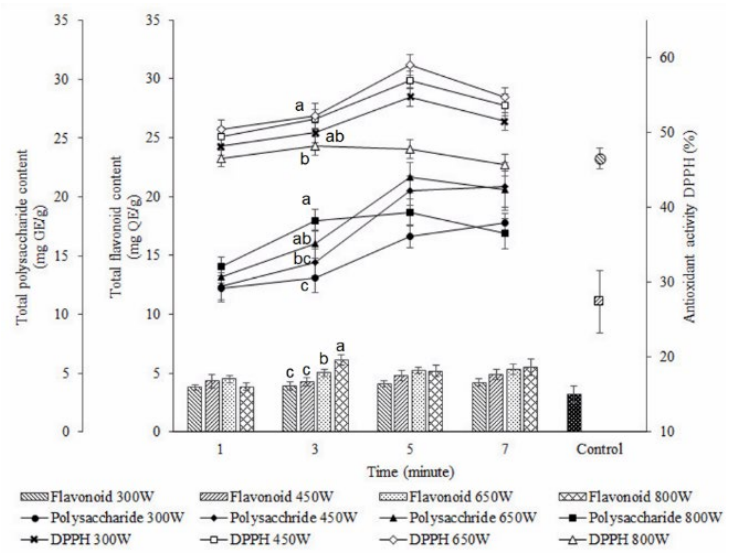

Figure 3. Effect of MAE on the total content of polysaccharides, flavonoids, and antioxidant activity

The use of microwave-assisted extraction in the extraction of bioactive ingredients from plants was reported in previous studies [17, 18]. The MAE process increases the internal temperature of the subject and accelerates cell wall disruption and disintegration, thereby enhancing extraction efficiency [19]. Our study results also showed that microwave power and time affected the efficiency of extraction of bioactive ingredients from agarwood leaves (Figures 1 and 2). When the MAE was at low power, more time was required for heating to the optimum extraction temperature (Figures 1 and 2). At low microwave power and sufficient time, the electromagnetic energy becomes concentrated and acts on the cell wall, increasing bioactive ingredient extraction efficiency [18, 20]. When the temperature reaches the optimum value, the extraction of bioactive substances, and the total contents of these ingredients, would increase [15]. At short microwave time, the content of the bioactive compounds significantly improved with increasing microwave power [18]. In our research with agarwood leaves, the short-term use of high-power microwaves resulted in the efficient extraction of bioactive compounds (Figures 1 and 2). However, previous studies noted that even at high microwave power (1000 W), the content of bioactive compounds was not increased compared to lower power for the same time of investigation [21]. This could be due to the higher power causing faster solvent movement speed and increased pressure, which accelerated the extraction process and led to the concentration of substances rapidly reaching equilibrium (Figures 1 and 2). Besides, increasing microwave power for a long time leads to an increase in temperature which can reduce the content of bioactive compounds [17]. The results obtained from the study showed that the bioactive compounds peaked under different conditions (Figures 2 and 3). The results suggested that based on the higher bioactive compounds obtained with shorter extracted time, the MAE with 800w for 3 min was an effective parameter for extracting bioactive compounds from agarwood leaves (Figures 2 and 3).

3.2 Effect of the ultrasound-assisted extraction on bioactive compounds from agarwood leaves

The use of UAE yielded higher results than the control samples (Figures 4 and 5). The results showed that ultrasound power and duration significantly affected (p<0.05) the total contents of polyphenols, polysaccharides, flavonoids, saponins, and DPPH antioxidant activity of agarwood leaf extracts. Within the same ultrasonic power, extracted bioactive compounds tended to increase with ultrasound time increased from 5 min to 10 min and reaching their highest values at this time. Considering the same ultrasound time (10 min), increase of ultrasonic power from 20% to 25% produced the highest total polyphenol content of 60.05±1.12 mg GAE/g sample, whilst increase of power up to 30% gave the highest polysaccharide content of 14.96±0.98 mg GE/g. Besides, total saponin content and DPPH antioxidant activity reached their highest levels of 246.49±3.25 mg OAE/g and 47.17±1.35%, respectively, when power was increased to 35%. Regarding flavonoid content, the bioactive compounds were stable during ultrasound treatment. The results also indicated that prolonging the ultrasound treatment time by more than 15 min led to significant decrease of bioactive compound contents.

The results obtained from the study illustrated the significant influence of ultrasonic power and time (p<0.05) on the total contents of bioactive ingredients extracted from agarwood leaves (Figures 4 and 5). Barba *et al*. [22] indicated that cells were disrupted by UAE, which allowed the solvent to penetrate more easily and quickly speeding up the extraction process. Each bioactive compound has a different optimum extraction temperature range. Therefore, when the power is increased, temperature also increases at a certain time of ultrasound, and the different ultrasonic powers yield different bioactive compounds [21, 23]. Wu *et al*. [24] observed that ultrasonic power and duration increase produces higher temperatures, and that maintenance of ultrasound at high temperatures and for a long time leads to biodegradation of bioactive compounds. Ultrasonic power greatly affects temperature of the sample solution. The higher the power, the higher the temperature

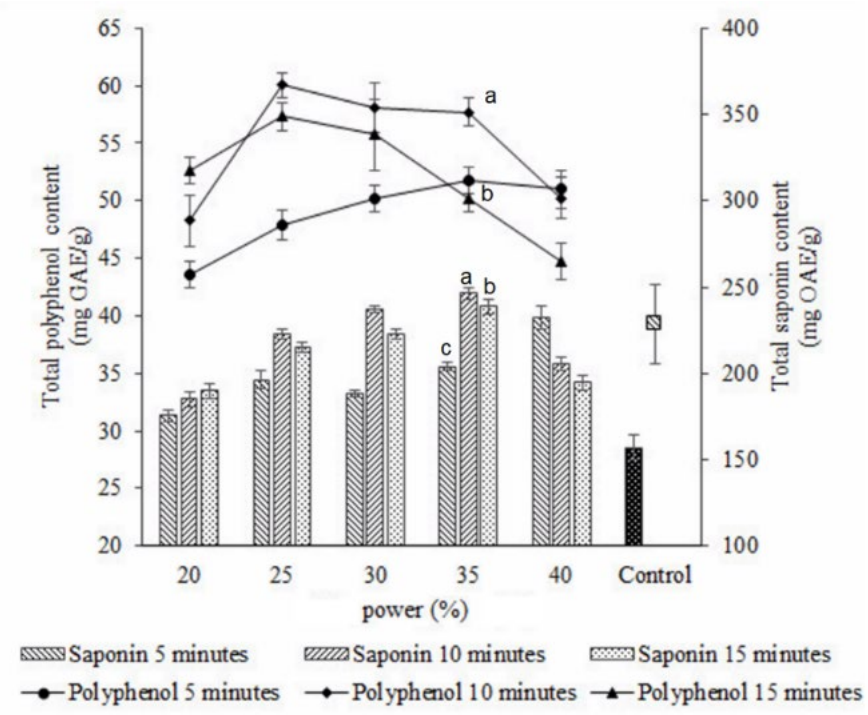

Figure 4. Effect of UAE on the total content of polyphenol and saponin

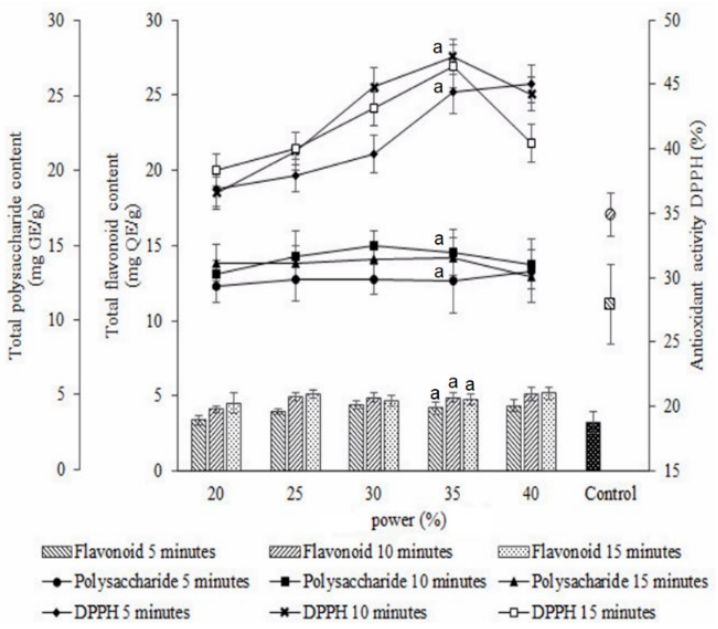

Figure 5. Effect of UAE on the total content of polysaccharides, flavonoids, and antioxidant activity

rises, which directly affects the content of bioactive compounds. With increased ultrasound power, stronger sonic erosion causes cell wall breakage, leading to increased levels of extracted bioactive compounds [23]. However, high temperatures can also reduce surface tension and increase pressure on the extracted mass, causing a decrease in extraction yield due to content loss [23]. The observation suggests that a shorter time is required to extract bioactive ingredients at high ultrasonic power. In agarwood leaves, the use of high power ultrasound with a shorter duration gave better extraction efficiency than low power use for a longer time (Figures 4 and 5). The present study showed that the ultrasound treatment at power of 35% of maximum capacity and duration of 10 min were the appropriate parameters for extracting bioactive compounds from agarwood leaves (Figures 4 and 5).

3.3 Effect of Viscozyme L treatment on bioactive compounds from agarwood leaves

The results from the study showed that Viscozyme L treatment significantly affected total polyphenols, polysaccharides, saponins, flavonoid contents, and DPPH antioxidant activity of agarwood leaves (Figures 6 and 7). Enzyme concentration and incubation time showed different effects on the total contents of these components (p<0.05). Considering at the same time-point of 6 h, the total contents of polyphenols, flavonoids, and polysaccharides were significantly affected (p<0.05) when the Viscozyme L concentration was increased from 1% to 3.5% (Figures 6 and 7). Specifically, the total contents of polyphenols and flavonoids increased rapidly when the Viscozyme L concentration was increased from 1% to 2.5% (from 45.92±2.88 mg GAE/g sample to 51.81±2.65 mg GAE/g sample and 4.24±0.47 mg QE/g of sample to 7.90±0.72 mg QE/g sample). When the enzyme concentration was increased, the content increased without any significant difference (p>0.05). Similarly, total polysaccharide content increased steadily and reached the highest value at 2.5% (23.711±1.26 mg GE/g sample) within 6 h (Figure 7). At the time of 3 h, the DPPH values increased steadily and reached the highest value at the enzyme concentration of 2% showing inhibitory concentration of 56.66±1.47%; saponin content increased to its highest level at 12 h with 443.587±5.02 mg OAE/g sample. It then decreased when the enzyme concentration was increased (Figure 7).

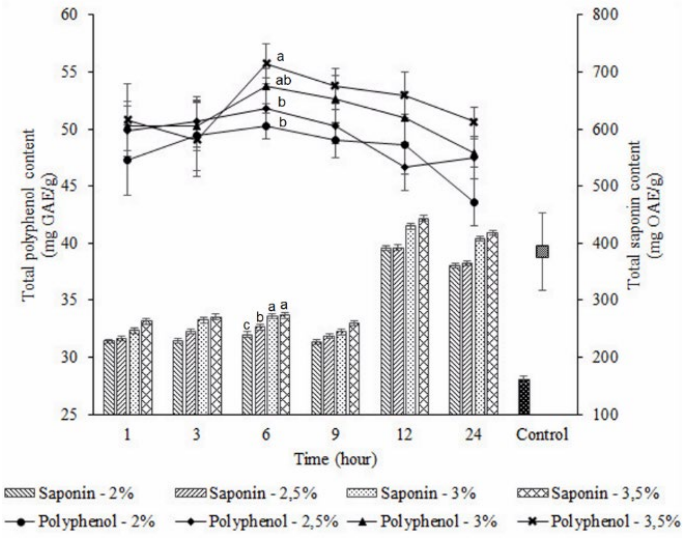

Figure 6. Effect of Viscozyme L on the total contents of polyphenols and saponins

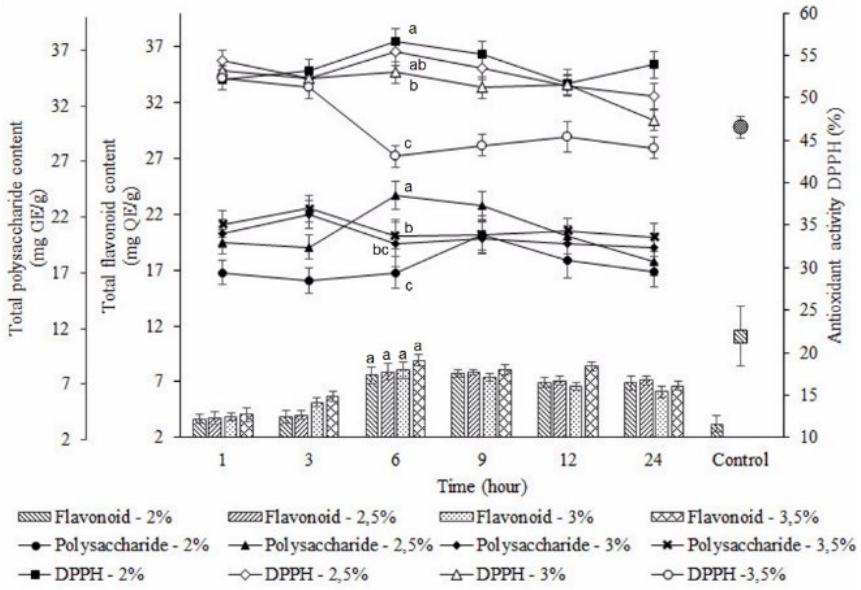

Figure 7. Effect of Viscozyme L on the total contents of polysaccharides, flavonoids, and antioxidant activity

The use of enzymes for bioactive compound extraction was reported in previous studies [10, 25]. The present study showed that the bioactive content varied significantly with different concentrations of Viscozyme L and incubation time (p<0.05). Enzymes bind to substrates to create enzyme-substrate complexes, disrupting bonds in the substrate [26]. Depending on the individual enzyme's ability, cell wall structural breakdown would also vary. When cell walls are hydrolyzed, bioactive substances are released [27] and are easier to exploit. During the same incubation period, an increase in the Viscozyme L concentration led to an increased reaction rate and more intense cleavage, thereby releasing bioactive compounds [10]. In our studies, the polyphenol content increased after 6 h of enzyme incubation and then decreased when the incubation period was extended (p<0.05) (Figure 6). Li *et al*. [25] suggested that when cell walls are cleaved, proteins in the cells are also released over time, and complexes with polyphenols cause a decrease in content. Initially, high substrate concentration accelerates the reaction rate, resulting in increased bioactive ingredient content, followed by gradual substrate depletion and a slight rise in these components [26]. Besides, environmental conditions can influence the levels of biologically active compounds, resulting in a loss with extended incubation time. Our results suggest that enzyme treatment at the concentration of 2.5% for 6 h is a suitable method for extracting bioactive ingredients from agarwood leaves (Figures 6 and 7).

3.4 Effect of fermentation process on bioactive compounds from agarwood leaves

Lactic fermentation by *L. acidophilus* resulted in higher levels of bioactive compounds than in unfermented samples (Figures 8 and 9). The results showed that fermentation time and initial microbial concentration significantly affected (p<0.05) the total contents of polyphenols, polysaccharides, flavonoids, saponins, and the DPPH antioxidant activity of agarwood leaves. (Figures 8 and 9). Generally, the total content of bioactive compounds and *L. acidophilus* biomass increased with fermentation time of up to 12 h and decreased with a further increase in fermentation

time (Figures 8, 9, and 10). With an initial concentration of microorganisms of 6 log CFU/mL, the total contents of polyphenols, polysaccharides, and saponins tended to increase with fermentation time and reached their highest level at 24 h (Figures 8 and 9). With increase of the initial concentration to 7 or 8 log CFU/mL, the total contents of polyphenols, polysaccharides, and saponins increased gradually and reached their highest values after 12 h of fermentation. The density of *L. acidophilus* increased with increase of bioactive ingredients at initial fermentation concentration of 7 log CFU/mL (Figure 10). At 12 h of fermentation, the total polyphenols and polysaccharide contents reached their highest values of 57.99±1.21 mg GAE/g and 17.74±0.88 mg GE/g in the case of 7 log CFU/mL of initial concentration, and total saponin contents showed the highest value of 251.02±3.22 mg OAE/g sample in the case of 8 log CFU/mL initial concentration. The results obtained from the study also indicated that the levels of these ingredients tended to decrease after 12 h of fermentation (Figures 8 and 9).

Figure 10 indicates that the density of *L. acidophilus* depended on initial density and time fermentation. Generally, biomass tended to increase during the fermentation process. The density of *L. acidophilus* peaked at 6 h in the case of 8 log CFU/mL concentration and 12 h in the case of 6 and 7 log CFU/mL concentrations. Fermentation causes complex matrix degradation or biological transformation into compatible components, thereby adjusting product properties or altering quantities of specific bioactive compounds [28]. During fermentation, various enzymes synthesized from bacteria act on the cell walls, breaking down peptide bonds and thereby causing release of bioactive compounds [29, 30]. Thus, these enzymes helped to increase the amounts of bioactive compounds released into the medium. However, when the fermentation time is prolonged, more organic acids are produced, and these acids inhibit metabolic processes, reducing the number of microorganisms [31]. The content of bioactive ingredients in this study tended to decrease with fermentation time (Figures 8 and 9). This could be due to the bioactive compounds, after being released from cells by the fermentation process, were then affected by temperature, light, and external environment, and were oxidized. Furthermore, the presence of lactic acid bacteria contributed to simple phenolic conversion and reduction of phenolic compounds of high molecular weight, decreasing the content of bioactive substances during fermentation [13]. The flavonoid content tended to decrease during fermentation, but this reduction was not significant (p<0.05) (Figure 9). A previous study also reported reduced flavonoid content during fermentation [32]. This could be due to the breakdown of flavonoid compounds with fermentation time. The results indicated that the fermentation process influenced the bioactive compounds, and an initial increase in biomass concentration contributed to a shorter fermentation duration (Figures 8, 9, and 10). The present study suggests that an initial concentration of microorganisms of 7 log CFU/mL and fermentation time of 12 h are suitable parameters for the extraction of bioactive ingredients from agarwood leaves and ensure the highest concentration of *L. acidophilus* (Figures 8, 9, and 10).

A comparison of the bioactive ingredient extraction efficiencies of the microwave, ultrasonic, Viscozyme L, and fermentation treatments for agarwood leaves is shown in Table 1. The results showed that bioactive compound extraction efficiency differed depending on the treatment method. Viscozyme L treatment showed to be the most effective method, followed by MAE and lactic fermentation, with ultrasound treatment showing the lowest result. Enzyme treatment was more effective than other treatments for extraction of total polysaccharides, flavonoids, saponins, and antioxidant activity (Table 1). However, the total polyphenol content of enzyme treatment was significantly lower than the content from other treatments.

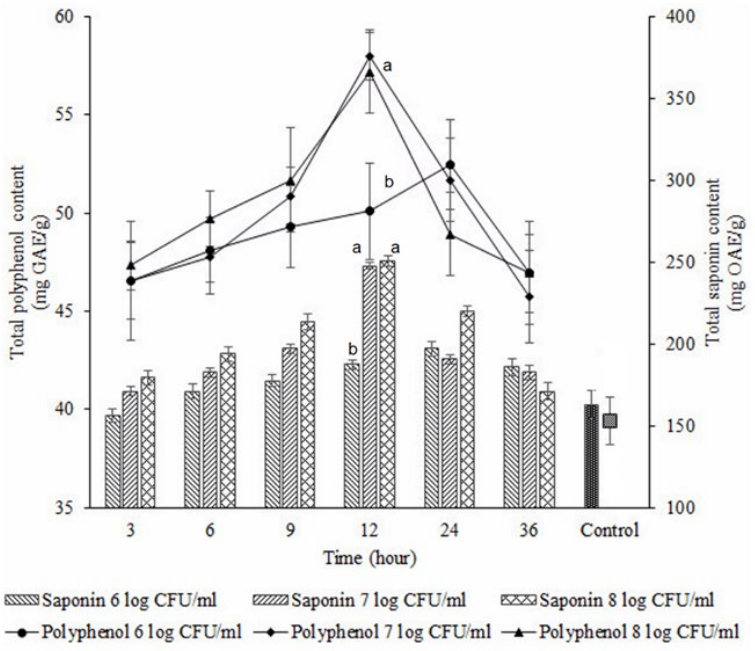

Figure 8. Effect of fermentation on the total contents of polyphenol and saponin

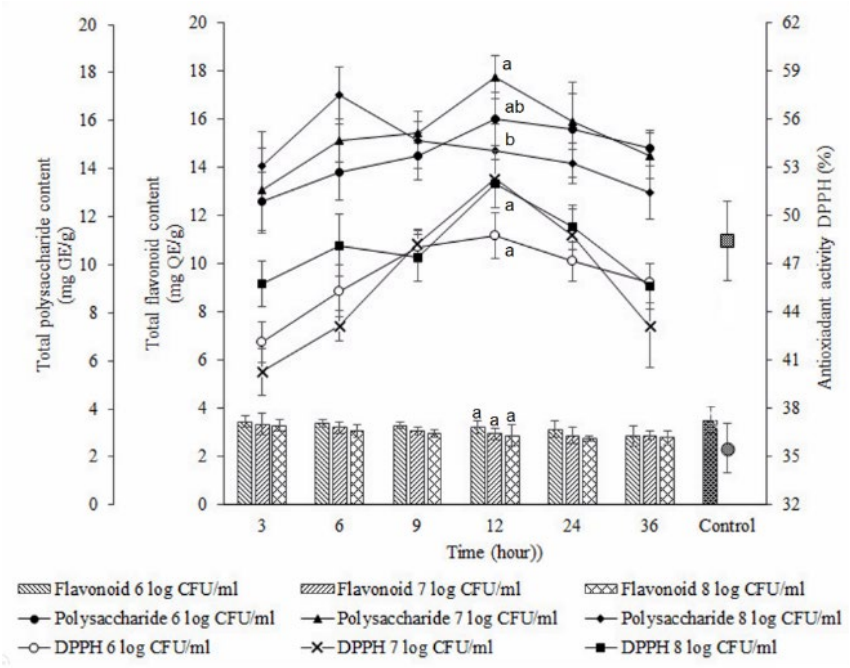

Figure 9. Effect of fermentation on the total contents of polysaccharides, flavonoids, and antioxidant activity

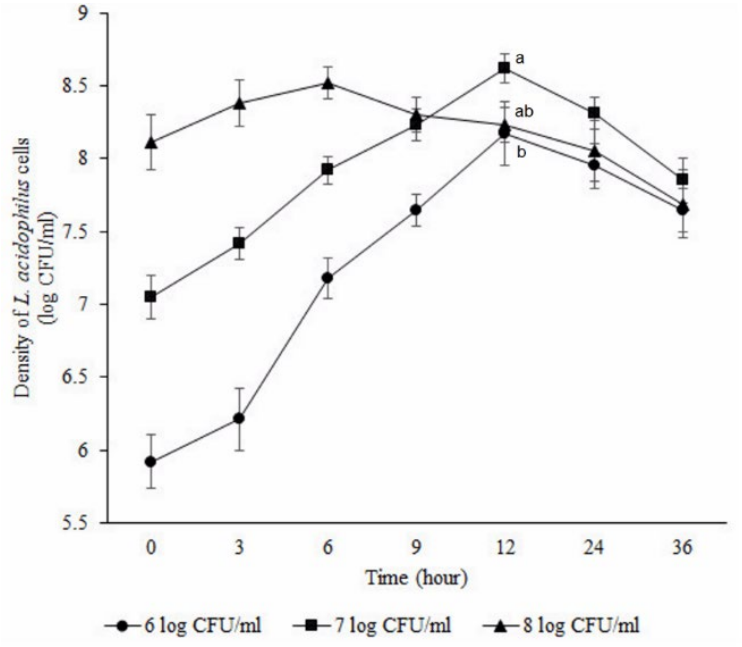

Figure 10. The viability of *L. acidophilus* during lactic fermentation

Table 1. Comparison of extraction methods

Bioactive Compounds	**MAE (800W; 3 min)**	**UAE (35%; 10 min)**	**Fermentation (10^7 CFU/mL; 12 h)**	**Viscozyme L (2,5%; 6 h)**
Total polyphenols content (mg GAE/g sample)	(59.28±1.68)^a	(57.70±1.21)^a	(57.99±1.21)^a	(51.81±2.65)^b
Total polysaccharide content (mg GE/g sample)	(17.95±0.92)^b	(14.51±1.54)^c	(17.74±0.88)^b	(23.71±1.26)^a
Total flavonoid content (mg QE/g sample)	(6.15±0.44)^b	(4.88±0.33)^c	(2.92±0.24)^d	(7.90±0.72)^a
Total saponins content (mg OAE/g sample)	(251.16±3.76)^a	(246.49±3.25)^a	(247.76±2.17)^a	(252.77±4.32)^a
Antioxidant activity (% inhibition)	(48.14±1.23)^c	(47.17±1.35)^c	(52.25±1.76)^b	(55.40±1.56)^a

Note: a, b, c: show significant differences by row ($p < 0.05$).

In previous studies, MAE treatment improved the extracted efficiency of polyphenols from tea leaves [20]. Similarly, UAE treatment impacted mulberry leaf cell walls, enhancing polysaccharide extraction effectively [23]. Besides, enzyme action significantly improved the content of bioactive ingredients extracted from brown algae [10]. Fermentation was shown to enhance the extraction of bioactive components from soybeans [29]. Similarly, lactic fermentation by *Lactobacillus plantarum* and *L. acidophilus* was also shown to improve bioactive compound content from *Aquilaria* spp. and *Anoectochilus formosanus* Hayata, respectively [11, 33]. These methods significantly improved the extraction efficiency of bioactive compounds. A comparison of the extraction efficiency of bioactive compounds from Ngoc Linh ginseng callus by UAE, MAE, and amylase enzyme methods showed that the enzyme method was the most efficient extraction technique for polyphenols and polysaccharides [34]. Besides, a comparison study of MAE, UAE, and lactic fermentation for extraction of bioactive compounds from *Anoectochilus formosanus* showed no significant difference (p>0.05) between these treatment methods [35]. The results indicate that the extraction efficiency of bioactive compounds from plants depended on extraction method and type of plant. However, the evaluation of the effect of these methods on agarwood leaves remained to be fully explored. The present study showed that microwaves, ultrasound, enzymes, and fermentation influence the bioactive compounds from agarwood leaves (Table 1). Though the polyphenol content in the case of enzyme treatment was the lowest, the antioxidant activity (DPPH) was the highest, followed by lactic fermentation. The MAE and UAE treatments showed the lowest antioxidant activity (%) values (Table 1). Antioxidant activity (DPPH) assay is one of the indicators used to evaluate antioxidant effectiveness. Other methods involve assay of reducing power, scavenging effect against hydrogen peroxide, and scavenging against superoxide anion radicals, etc. [11]. In the present study, the MAE and UAE treatments caused overheating inside the medium, affecting the antioxidant activity of compounds (Table 1). Besides, the results indicate that excessive prolonging of fermentation time also affected the sensitive compounds with antioxidant activity released from plant cells. The MAE and UAE treatments improved the bioactive compounds with short treatment time. Enzyme treatment required more time of treatment than MAE and UAE treatments but showed the highest efficiency. The lactic fermentation process took the most time to reach extraction efficiency. Besides the evaluated compounds, the lactic fermentation process can create other valuable compounds and natural antimicrobial compounds. Also, lactic fermentation can provide beneficial bacteria sources. The combination of these extraction methods should be evaluated in further study to evaluate the extraction efficiency of bioactive compounds from agarwood leaves.

4. Conclusions

The results obtained from the present study showed that treatment with MAE, UAE, Viscozyme L, and fermentation facilitated the extraction of bioactive compounds from agarwood leaves. The extraction efficiency of the bioactive compounds differed with treatment methods. Viscozyme L treatment proved to be the most effective method, followed by MAE treatment, lactic fermentation, and UAE. The results also indicate that MAE and UAE treatments caused overheating inside the medium, leading to the effect on the antioxidant activity compounds present, whereas prolonged fermentation time also affected the sensitive antioxidant released from the plant cells. The lactic fermentation process required the most time to reach extraction efficiency. However, fermentation can provide valuable metabolic products and could be a source of beneficial bacteria. Agarwood leaf fermentation would be a potential approach to provide bioactive compounds and probiotics for health benefits including food supplementation.

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