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Ecological Condition, Phytochemical Screening, and Antioxidant Potential of Karas (Aquilaria malacensis L.) Leaves under Different Plant Conditions

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Abstract

The karas tree (Aquilaria malaccensis L.) is an agarwood-producing plant with health benefits. Its leaves contain bioactive compounds that contribute to antioxidant activity. Ecological factors, including soil, climate, and biological conditions, influence the production of these secondary metabolites. Identifying ecological conditions that enhance antioxidant activity is important for optimizing karas leaves as a herbal medicine. This study aimed to determine the ecological characteristics affecting the antioxidant activity of karas leaves. A survey using purposive sampling was conducted, involving three plant categories: induced plants with agarwood formation, induced plants without agarwood formation, and non-induced plants without agarwood formation. Quantitative data were analyzed using an independent t-test with unequal sample sizes at the 5% significance level. Rainfall, altitude, light intensity, chlorophyll content, ash content, and crude fat were relatively similar among samples, whereas soil nutrient conditions and agarwood formation significantly affected the responses. Antioxidant activity was classified as very strong in all samples, with IC50 values of 32.215 ppm in induced plants with agarwood formation, 32.814 ppm in induced plants without agarwood formation, and 14.629 ppm in non-induced plants without agarwood formation. Non-induced plants without agarwood formation differed significantly from the other categories, whereas induced plants with and without agarwood formation did not differ significantly. Phytochemical screening detected alkaloids, flavonoids, phenols, tannins, and triterpenoids/steroids in all samples. These findings indicate that ecological conditions and agarwood formation influence antioxidant activity and crude fat content in karas leaves. Nevertheless, all samples retained very strong antioxidant activity, confirming their potential as a natural source of herbal medicine.

Keywords: agarwood, padang city, secondary metabolites, stress

1. Introduction

Agarwood is one of the Non-Timber Forest Products (NTFPs) with high economic and medicinal value. One plant species that produces agarwood is karas (Aquilaria malaccensis L.), a member of the Thymelaeaceae family. It is one of the species that produces agarwood. This plant is also known locally as tengkaras, kayu karas, or gaharu, while internationally it is referred to as agarwood, aloewood, or eaglewood. Karas naturally occur across the Sumatra and Kalimantan islands. According to the International Union for Conservation of Nature (IUCN), it is classified as critically endangered due to overexploitation, habitat conversion, and limited cultivation (Yuwono et al., 2021). In addition to agarwood production, the leaves of Aquilaria malaccensis also have medicinal and pharmacological properties, including antioxidant activity (Mokhtar et al., 2021; Hamzah et al., 2024; Ghiffari & Fevria, 2024).

Karas leaves have been reported to contain secondary metabolites with important antioxidant functions such as phenols, terpenoids, flavonoids, glycosides, tannins, and steroids/triterpenoids (Ghiffari & Fevria, 2024; Ridwanto et al., 2017), with very strong antioxidant activity (Hasanah et al., 2020; Batubara et al., 2019). As karas leaves are abundantly available, their potential use as herbal medicine offers a sustainable alternative for utilizing non-resinous karas trees without cutting them down (Wangiyana et al., 2019). This is important because agarwood formation takes a long time, and not all trees can be naturally infected. Understanding the ecological factors influencing the antioxidant activity of karas leaves will enhance their effective utilization.

Antioxidants function as cellular defenses against damage caused by free radicals through neutralizing oxidative reactions in the human body. Although humans possess natural antioxidant defense systems as part of metabolism, excessive free radical production can overwhelm this system, leading to oxidative stress and cellular damage. In such cases, external (exogenous) antioxidants from natural sources, such as medicinal plants like karas leaves, are needed.

Unfavorable ecological conditions can trigger plant cells to synthesize secondary metabolites as physiological responses in defense against pests, pathogens, allelopathic interactions, and other stresses. Ecological factors are divided into biotic and abiotic. Biotic factors include microorganisms such as pathogenic fungi and bacteria, whereas abiotic factors include soil chemistry, climate, temperature, and humidity. The accumulation of secondary metabolites in karas leaves is strongly influenced by these external factors. According to Gardner (1991), external factors affecting plant growth include climate, edaphic (soil) conditions, and biological aspects. These factors not only influence plant growth and development but also determine the patterns and levels of secondary metabolite accumulation. Since secondary metabolites are synthesized from primary metabolic precursors, environmental changes can alter both the quantity and diversity of active compounds.

The karas leaves used in this study were collected from trees approximately 15 years old, which were grown under natural field conditions without fertilization, pruning, or other intensive management practices. At around 10 years of age, the trees were subjected to artificial wounding with rusty nails to induce biotic stress and stimulate agarwood formation. Subsequently, initial signs of resin development were observed between 13 and 14 years of age. At 14 years, additional induction was performed through repeated wounding and microbial inoculation; however, the specific microorganisms used were not disclosed by the field operator and therefore could not be identified or characterized in this study (Nurhayati, personal communication, 2025).

The use of rusty nails causes mechanical injury, which serves as a primary trigger for agarwood induction by disrupting plant tissues and potentially facilitating the entry of naturally occurring endophytic or pathogenic microorganisms through the wound sites. This process may lead to a combined response of abiotic stress (mechanical injury) and biotic interactions (microbial colonization), which together contribute to resin (agarwood) formation. Under these conditions, the induced trees exhibited typical agarwood-associated characteristics, including partial leaf shedding, the development of a distinct agarwood aroma, and the formation of dark brown resinous deposits in the cambial region (Aqmarina, 2018). Although other environmental factors, such as soil conditions, temperature, and microclimate, may also influence plant growth and secondary metabolite production, these factors were not experimentally controlled in this study and are assumed to be relatively uniform within the study area. Therefore, the observed agarwood formation is primarily attributed to the applied wounding treatment, in combination with possible natural microbial colonization at the wound sites, rather than being solely driven by a single inducing factor.

This background underlies the present study, which aims to compare the antioxidant activity of karas leaves from trees exposed to different ecological conditions. Based on the literature review, studies examining the antioxidant potential of karas leaves under specific environmental conditions, such as climate, temperature, humidity, rainfall, and soil chemistry/physics, remain limited. Therefore, this research was conducted under the title: "Ecological Condition, Phytochemical Screening, and Antioxidant Potential of Karas (Aquilaria malaccensis L.) Leaves under Different Plant Conditions".

2. Materials and Methods

This research was conducted from April to July 2025. Experimental materials were collected from

Aquilaria malaccensis L. (karas) trees planted in Jl. Air Dingin, Balai Gadang, Koto Tangah District, and at Andalas University Campus, Limau Manis, Pauh District, Padang City. The preparation of simplicia and extraction were carried out at the Plant Physiology Laboratory, Faculty of Agriculture UNAND; phytochemical screening was conducted at the KOBA Laboratory, Faculty of Mathematics and Natural Sciences UNAND; proximate analysis was conducted at the Animal Biotechnology Laboratory, Faculty of Animal Science, UNAND; soil analysis was performed at PT. Wiwiadi Bintang Sains Laboratory, while antioxidant activity analysis was carried out at the LLDIKTI Region X Laboratory, West Sumatra Province.

This study employed a survey method with purposive sampling, using three criteria for leaf samples: induced with agarwood formation (A1), induced without agarwood formation (A2), and non-induced without agarwood formation (A3). The repetitions for each sample A1, A2, and A3 are 2,3, and 3, respectively. Thus, the total sample population used is 8. The number of replications per sample group varied due to the limited field availability of plant material.

The parameters observed in this study included soil analysis, rainfall data, and climate classification, chlorophyll content, percentage of extract yield, Phytochemical screening, proximate analysis, and antioxidant activity (IC_{50}). Several parameters are observed simultaneously. The variable soil analysis is described by Eviati (2024). Soil samples were collected from the canopy area of Aquilaria malaccensis at two locations, namely Koto Tangah District and Pauh District. At each site, two sampling points were selected diagonally around the canopy and composited to represent the same area. Soil samples were collected using a Belgian auger at 0–20 cm depth, followed by the removal of roots and weeds. The samples were then air-dried and sieved through a 5-mesh sieve to obtain a homogeneous soil fraction. Climate data were obtained from secondary sources provided by the West Sumatra Climatology Station, based on daily observations that were averaged to represent climatic conditions during the study period.

Chlorophyll content was measured concurrently during the collection of A. Malaccensis leaves are used for other analyses. Five leaves from each sample were randomly selected from leaf positions three, counted from the shoot tip. The chlorophyll content was determined using a chlorophyll meter and expressed in SPAD units. Chlorophyll content was measured at three leaf positions (basal, middle, and apical), and the mean value was calculated.

The observed variables included phytochemical screening and antioxidant activity analysis of the viscous extract from A. Malaccensis leaf simplicia. Karas leaves were collected from each predetermined sample with a total fresh weight of 1 kg. The selected leaves met the criteria of being green and not shaded. Leaves were collected from positions 1–4 from the shoot tip. The collected leaves were cleaned to remove adhering impurities before drying. Drying was performed by placing the leaves on aluminum foil and heating them in an oven at 60 °C for 48 hours. After drying, the leaves were ground using a blender and subsequently sieved to obtain a fine simplicia powder.

The powdered simplicia of A. Malaccensis leaves (30 g) were extracted by maceration using 96% ethanol at a 1:10 (w/v) ratio for 24 h at room temperature in the dark. The extraction was repeated for three days, and all filtrates were combined. The solvent was removed using a rotary evaporator, followed by a water bath at 60°C to obtain a viscous extract.

Screening was conducted to identify the presence of major secondary metabolites in the Viscous leaf extracts. Proximate analysis was performed to determine the nutritional composition of the samples, including moisture, ash, crude protein, crude fat, and carbohydrate content, according to AOAC (2012) standard procedures.

Antioxidant activity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method. A DPPH solution (100 ppm) was prepared in methanol p.a., and the mixture was incubated for 30 min to determine the maximum absorbance. The viscous extract of Aquilaria malaccensis leaves was prepared as a 1000 ppm stock solution and diluted to obtain five concentration variations, namely 10, 20, 30, 40, and 50 ppm for samples A1 and A2, and 3, 6, 9, 12, and 15 ppm for sample A3. Each sample solution was mixed with DPPH and incubated at room temperature for 30 min. Absorbance was measured using a UV–Vis spectrophotometer at 516 nm. Ascorbic acid (2 ppm, 2.5 ppm, 3 ppm, 3.5ppm, 4 ppm) was used as a positive control, and antioxidant activity was expressed as percentage inhibition. The IC_{50} value was determined from the linear regression equation ($y = ax + b$) obtained from the relationship between sample concentration and percentage inhibition. The IC_{50} value was calculated using the equation $IC50=(50-b)/a$.

Data obtained from chlorophyll content, extract yield percentage, IC_{50} values, and proximate analysis were statistically analyzed using an independent t-test at a 5% significance level. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) to compare the two groups.

3. Results and Discussion

Soil analysis was conducted in two sampling areas in this study: Koto Tangah District (A1 and A2) and Pauh District (A3). The results of soil analysis for several variables from the two sampling areas are presented in Table 1.

Table 1. Soil Analysis of Pauh District and Koto Tangah District

Soil Parameter	Methode	Location	
		Koto Tangah District	Pauh District
N Total (%)	Detector nutrient Analyzer C-3000 RS-485	0.16 (L)	0.09 (VL)
P2O5 (ppm)		6.82 (L)	4.92 (VL)
K- exc (cmol/kg)		0.19 (L)	0.08 (VL)
Al- exc (cmol/kg)	KCl Extraction 1N	2.98 (L)	4.11 (H)
Fe-exc (ppm)	Morgan dan Wolf	19.51 (L)	34.00 (H)
C-Organic (%)	Walkey dan Black	1.18 (L)	0.88 (VL)
pH H2O	electrometric	5.54 (A)	4.93 (A)
Texture	Hidrometer 152 H	Clay	Clay
Soil order	Secondary data	Inseptisol	Ultisol

Note: VL (very low); L (Low); H(high); A(Acid). For assessing soil analysis results, refer to Eviati et al. (2024).

Soil samples were collected at 0–20 cm depth around the canopy of the sample plants in both districts. The chemical properties of the soil in Pauh District, including organic C, total N, available P_2O_5, and K- exc, were categorized as very low. In contrast, in Koto Tangah District, these elements were classified as low. Al-exc and Fe-exc were classified as high in Pauh District, whereas both Al-exc and Fe-exc were classified as low in Koto Tangah District. Despite these differences, the soils at both locations were acidic. The observed variation in soil chemical properties is likely associated with differences in soil order, as ultisols are generally more acidic than inceptisols.

The results of this study are consistent with the findings of Gogoi (2021), who examined the relationship between soil quality and naturally formed agarwood in Aquilaria trees, reporting that low pH, fine soil texture, and high organic C content play crucial roles in natural agarwood infection. In contrast, the soil analysis in this study showed that the chemical status of soils in Pauh District was poorer than in Koto Tangah District, with very low levels of N, available P_2O_5, exchangeable K, and organic C. Meanwhile, the soil nutrient levels in Koto Tangah District were categorized as low. Inceptisols dominate the Koto Tangah area, whereas Ultisols characterize Pauh. These soil chemical properties are closely related to organic C levels; the lower the organic C content, the lower the values of other chemical parameters, and vice versa. Based on the observed nutrient status, it is highly unlikely that Aquilaria trees will naturally produce agarwood under these conditions.

However, in this study, the presence of agarwood in trees cultivated in Koto Tangah District (A1) was due to artificial wounding and pathogenic fungal induction. Nevertheless, the success of agarwood formation under induction might also be influenced by the presence of mycorrhizae, fungi, and bacteria, which play important roles in agarwood formation in Aquilaria, as acidic soil conditions may enhance fungal survival.

Weather conditions and climate types of the two sampling sites are presented in Table 2. It can be seen that both Koto Tangah and Pauh share similar weather and climate types, indicating that these factors did not affect the subsequent variables measured in this study.

Table 2. Monthly Rainfall Data (April–June 2025) in Koto Tangah and Limau Manis Districts, Padang City

Sampling Area (District)	Month	Parameter			
		Total Rainfall	Number of Rainy Days	Number of Dry Days	Oldeman Climate Type
Koto Tangah (A1 & A2)	April	421.9	19	11	Type A
	May	190.3	8	23	
	June	279.3	11	19	
Average		297.1	13	18	
Pauh (A3)	April	424	19	11	TypeA
	May	234.5	10	21	
	June	228.5	8	22	
Average		295.66	12	18	

Source: Secondary data from the West Sumatra climatology station 2025

Table 3. Extraction yield percentage, Chlorophyll content, and Elevation

Sample	**Average**		**Elevation**
	Extraction Yield (%) ± SD	**Chlorophyll (SPAD unit)± SD**	**(m a.s.ll)**
Induced, with agarwood formation (A1)	16.789 ± 1.639	59.050 ± 10.074	61.36
induced, without agarwood formation (A2)	38.717 ± 21.647	67.926 ± 0.838	61.36
non-induced, without agarwood formation (A3)	56.339 ± 9.071	68.052 ± 9.189	243,43
T-test	**t-value**	**t-value**	**Elevation category**
A1 VS A2	1.747 ns	-1.261 ns	Low
A1 VS A3	-7.374 *	-0.99 ns	Low
A2 VS A3	-1.300 *	0.024 ns	Low

*Note: Different letters indicate significant differences at the 5% level. *significantly different at the 5% level, ** indicates highly significant differences at the 1% level, and ns indicates no significant difference.*

The analysis of the percentage yield of the viscous extract using a t-test at the 5% significance level showed that sample A3 was significantly different from A1 and A2, whereas A2 was not significantly different from A1. The non-induced sample without agarwood (A3) produced the highest yield at 56%. This sample was collected from an area at approximately 243 m above sea level. The data are presented in Table 3.

The percentage yield in this study was higher than in previous studies (Hasanah et al., 2020; Suhardiman & Juanda, 2019; Suharti et al., 2014) conducted in lowland, midland, and highland areas. In earlier research using the same maceration extraction method with 96% ethanol as a solvent, Suharti et al. (2014) reported a yield of 11.02% from Aquilaria malaccensis leaves collected in Bukit Sundi Sub-district, Solok Regency, at an altitude

of approximately 600 m above sea level (midland). Hasanah et al. (2020) reported a yield of 3.57% from leaves collected in Belitang, South Sumatra, at an altitude of approximately 65 m above sea level (lowland), which was still lower than the yields of samples A1 and A2 in this study, collected from trees at around 61 m above sea level. Furthermore, Suhardiman & Juanda (2019) obtained 9.044% from leaves collected in Majalaya, Bandung Regency, at an altitude of about 600 m above sea level (highland).

Previous studies (Hasanah et al., 2020; Suhardiman, 2023; Suharti et al., 2014) did not provide details on the physiological conditions of the sampled trees, making direct comparison with the present study difficult. Nevertheless, the relatively high extract yield observed here is likely due to the condition of the sampled trees, particularly the non-induced, non-agarwood sample (A3), which yielded the highest percentage (56.339%), followed by the induced, non-agarwood sample (A2, 38.717%), and the induced, agarwood-forming sample (A1, 16.789%). One limitation of the maceration method is that only certain active compounds are extracted into the solvent, which may influence the quantity of antioxidant compounds obtained (Senapati dan Behera, 2023).

Chlorophyll plays a central role in photosynthesis by producing glucose as a primary metabolite, which is subsequently synthesized into carbohydrates, lipids, and proteins. The chlorophyll content showed no significant differences among the samples, as determined by a t-test at the 5% significance level. The chlorophyll content obtained in this study ranged from 59.050 to 68.52 SPAd units. The chlorophyll content obtained in this study was higher than that reported by Kaewprayoon et al (2015), who observed that the chlorophyll content of Aquilaria crasna under fertilization treatment ranged from 36.7 to 58.0 SPAD units. Similarly, the chlorophyll content of other tree species, including Quercus, Prunus, and Hopea, has been reported to range between 10 and 60 SPAD units (Zhu et al., 2012).

Chlorophyll content is regulated by the interaction of multiple environmental and physiological factors rather than by the availability of a single nutrient. Recent reviews have demonstrated that chlorophyll metabolism is simultaneously influenced by light intensity, temperature, water availability, altitude, oxygen status, and mineral nutrients, particularly nitrogen, magnesium, and Fe, through coordinated regulation of chlorophyll biosynthesis and degradation (Yong, 2024).

Nitrogen and Fe are essential nutrients involved in chlorophyll biosynthesis. Nitrogen constitutes a major component of chlorophyll molecules and photosynthetic proteins, whereas Fe functions as a cofactor for several enzymes involved in chlorophyll biosynthesis, even though Fe is not a structural component of the chlorophyll molecule (Yong, 2024). In this study, the two sampling sites exhibited contrasting nutrient availability. Soil from Koto Tangah contained low concentrations of both N and Fe, whereas soil from Pauh District showed very low N but high Fe. Thereby reducing the overall effect of individual nutrients on chlorophyll accumulation (Table 1).

Elevations were relatively comparable between the two sites, both of which are classified as lowland areas. This is further supported by the identical average number of dry days recorded at both locations (18 days) during the study period (Table 2), suggesting limited climatic variation between sites. Consequently, the relatively similar environmental conditions, together with the contrasting availability of nitrogen and Fe, may explain why chlorophyll content did not differ significantly between the two locations. However, because leaf nutrient concentration and nutrient uptake efficiency were not determined, this study cannot attribute the observed chlorophyll response solely to soil nitrogen, and nutrient analyses are required to clarify the mechanisms underlying chlorophyll accumulation in A malaccensis.

Proximate analysis (Table 4) showed variations in nutrient composition among the samples (A1, A2, and A3). The moisture content of induced karas leaf simplicia with agarwood formation (A1) was not significantly different from that of induced karas without agarwood formation (A2). However, both samples showed a significant difference compared with non-induced karas without agarwood formation (A3) at the 5% significance level. The moisture content of all samples met the Ministry of Health (Kemenkes, 2017) criteria for good-quality simplicia, which are set at below 10%. Lower moisture content is generally associated with better stability of bioactive compounds because enzymatic and microbial activities become more limited, thereby minimizing the degradation of antioxidant compounds (Shahidi & Zhong, 2015). This condition indicates that the non-induced karas leaf simplicia without agarwood formation (A3) potentially possesses better storage stability than the other samples. This may also influence antioxidant activity, as water can contribute to the oxidative degradation of bioactive compounds (Santoso et al., 2022).

Table 4. Proximate Analysis

Sample	Moisture content	Ash content	Crude fiber	Crude fat
	-----%----			
Induced, with agarwood formation (A1)	9.14 ± 0.057	5.7 ± 0.382	21.44 ± 0.113	2.4 ± 0.071
induced, without agarwood formation (A2)	9.07 ± 0.487	5.04 ± .599	18.837±0.176	3.01 ± 0.452
non-induced, without agarwood formation (A3)	7.43 ± 0.164	6.05 ± 1.021	18.81 ± 0.026	2.65 ± 0.254
t-test	**t value**			
A1 VS A2	0.247 ns	0.683 ns	20.158 **	-2.295 ns
A1 VS A3	16.661 **	-0.545 ns	32.292 **	-1.591 ns
A2 VS A3	5.533 *	-0.922 ns	0.260 ns	1.213 ns

*Notes: Different letters indicate significant differences at the 5% level. *significantly different at the 5% level, ** indicates highly significant differences at the 1% level, and ns indicates no significant difference. Induced, with agarwood formation (A1) and induced, without agarwood formation (A2) sample; District Pauh: non-induced, without agarwood formation (A3) sample.*

For the ash content variable, the results indicated no significant differences among the samples using a t-test at the 5% significance level. Ash content reflects the mineral composition within plant tissues, which functions as cofactors for antioxidant enzymes such as superoxide dismutase, catalase, and peroxidase. Minerals such as Zn, Cu, and Fe play important roles in the plant antioxidant defense system by facilitating the detoxification of free radicals generated during cellular metabolism (Broadley et al., 2012). Therefore, the relatively similar ash content suggests that the mineral-supported antioxidant capacity among the three samples does not differ substantially.

The crude fiber content of induced karas leaf simplicia with agarwood formation (A1) compared with induced karas leaf simplicia without agarwood formation (A2) and non-induced karas without agarwood formation (A3) showed a significant difference at the 5% significance level. However, the crude fiber content between induced karas without agarwood formation (A2) and non-induced karas without agarwood formation (A3) did not show a significant difference. The higher crude fiber content observed in samples A1 and A2 is presumably related to the physiological response of plants to the agarwood induction process, which shifts plant metabolism toward defense mechanisms and the production of secondary metabolites. The increase in crude fiber content may be associated with the accumulation of cell wall components such as cellulose, hemicellulose, and lignin. These structural components are often associated with bound phenolic compounds that possess antioxidant activity. Phenolic compounds bound to the lignocellulosic matrix can act as a source of antioxidants that may be released during extraction or digestion processes (Acosta-Estrada et al., 2014). In contrast, the high levels of N, P, K, organic C, as well as Al and Fe (Table 1) in the growing location of non-induced karas leaves without agarwood formation (A3) may suppress the lignification process

Meanwhile, the crude fat content of the three samples ranged from 2.40% to 3.01% and did not differ significantly according to a t-test at the 5% significance level. Lipids in plant tissues may serve as a medium for the accumulation of lipophilic antioxidant compounds, such as carotenoids and tocopherols, which protect cell membranes from oxidative damage. Lipid compounds are also associated with plant stress conditions that can stimulate increased production of secondary metabolites (Herms & Mattson, 1992).

Phytochemical screening was carried out as an initial step prior to antioxidant activity analysis, aiming to identify the chemical compound classes present in the leaf extracts of *Aquilaria malaccensis* under different treatments. The results of the phytochemical screening provide essential information on candidate compounds contained in *A. malaccensis* leaves. The findings are presented in Table 5.

Table 5. Qualitative Phytochemical Screening of Karas Leaf Extracts

Nama Senyawa	Induced, with agarwood formation		Induced, without agarwood formation			Non-induced, without agarwood formation		
	1	2	1	2	3	1	2	3
Alkaloid	+	+	+	+	+	+	+	+
Flavonoid	+	+	+	+	+	+	+	+
Fenol	+	+	+	+	+	+	+	+
Saponin	-	-	-	-	-	-	-	-
Tanin	+	+	+	+	+	+	+	+
Triterpenoid/steroid	+	+	+	+	+	+	+	+

Note: (+) indicates the presence of the compound; (−) indicates the absence of the compound.

The results of the phytochemical screening showed that the secondary metabolite content of each plant sample included alkaloids, flavonoids, phenols, tannins, and triterpenoids/steroids, all of which consistently tested positive (+) in each replicate. In contrast, saponins tested negative (–), indicating their absence in all samples. These components are generally known for their applications in medicine and nutrition (Julianto, 2019). The presence of these compounds in the viscous leaf extracts of *A. malaccensis* indicates that the samples possess antioxidant potential. The phytochemical screening results in this study are consistent with the findings of Ghiffari & Fevria (2024), although other studies have reported the presence of saponins in *A. malaccensis* leaves (Suhardiman, 2023; Santoso et al., 2022).

After confirming the presence of candidate compounds with potential antioxidant activity, the viscous extracts were further evaluated for their IC_{50} values. The t-test analysis at a 5% significance level showed that the IC_{50} value of sample A2 was significantly different from that of sample A3, but not significantly different from that of sample A1. In addition, the IC_{50} value of sample A1 was not significantly different from that of sample A3 according to the t-test at the 5% significance level. Among all treatments, sample A2 had the lowest IC_{50} value. The IC_{50} values are presented in Table 6 as mean ± standard deviation (SD). The SD values indicate the variability of the data, whereas the t-test results reflect differences in the means between the compared samples.

Table 6. Inhibitory Concentration (IC_{50}) Values of Aquilaria malaccensis Leaf Extracts

Sample	**IC50 Everage ± SD**	**Antioksidan Activity**
Induced, with agarwood formation (A1)	32.215 ± 10.444	Very high
induced, without agarwood formation (A2)	32.814 ± 8.787	Very high
non-induced, without agarwood formation (A3)	14.629 ± 6.011	Very high
Vit C	3.860	Very high
t-test	**t value**	
A1 VS A2	-.0669 ns	
A1 VS A3	2.155 ns	
A2 VS A3	2.959 *	

*Notes: * significantly different at the 5% level, ns indicates no significant difference. Negative value (-) indicates the difference between the two compared samples. IC50<50 μg/ml (very strong), 50-100 μg/ml (strong), 100-150 μg/ml (moderate), 150-200 μg/ml (weak), >200 μg/ml (very weak) (Molyneux, 2004).*

Various studies have demonstrated that stress treatments are widely applied to enhance the accumulation of secondary metabolites in plants. For instance, drought stress has been reported to increase secondary metabolite production in chili (Gulo et al., 2024; Mahmood et al., 2021), oil palm (Hardianti, 2023), tomato (Reimer et al.,2021), and other species. In *Aquilaria malaccensis* (karas), stress is deliberately applied to induce the formation of agarwood, which accumulates in the stem tissue. Both biotic and abiotic stresses are commonly used for this purpose.

Abiotic stress, such as planting karas on ex-mining soils in Sawahlunto, has been shown to trigger agarwood formation due to heavy metal exposure (Satria et al., 2025). However, it remains unclear whether the plants in such conditions are also naturally infected by pathogenic fungi that may contribute to the agarwood formation process. On the other hand, biotic stress is commonly applied through wounding of the stem. This practice exposes plant tissues, thereby facilitating pathogen entry and accelerating agarwood development.

Stem wounding not only increases the risk of infection but also induces physiological stress in the plant. In response, the plant attempts to heal the wound by producing secondary metabolites, namely phytoalexins, which are secreted as resinous exudates that later harden within the parenchyma tissues. This

defense mechanism, while effective in agarwood induction, also disrupts the translocation of water and nutrients to the leaves, ultimately influencing the production and accumulation of other secondary metabolites. Based on the IC_{50} values obtained, the use of Aquilaria malaccensis leaves as an antioxidant is more effective when using non-induced leaves without agarwood formation (A3), collected from leaf positions 1–4 from the shoot tip. The antioxidant activity of all samples was classified as "very strong." However, the viscous extract of non-induced leaves without agarwood formation (A3) exhibited a lower IC_{50} value of 14.629 ppm, equivalent to 0.01125 mg of viscous extract. This indicates that, at a relatively low concentration, sample A3 inhibited 50% of free radical oxidation reactions. This finding is associated with the relatively high extract yield observed in sample A3.

It can be inferred that stress conditions in *A. Malaccensis*, such as stem wounding and agarwood formation, may reduce the accumulation of secondary metabolites in leaves, as the plant reallocates metabolic resources to the stem, the primary site of metabolite production. The results of this study, based on the IC_{50} value of the ethanol extract from non-induced A. Malaccensis leaves without agarwood formation (A3), showed better antioxidant activity than that reported by Nugraha et al. (2015), which reported an IC_{50} of 28 ppm

4. Conclusions

The concentrated leaf extract of *Aquilaria malaccensis* (karas) contains secondary metabolites such as alkaloids, flavonoids, phenols, tannins, and triterpenoids/steroids, while saponins were not detected in any of the samples. All three types of samples induced with agarwood formation (A1), induced without agarwood formation (A2), and non-induced without agarwood formation (A3) showed potential as antioxidants with very strong activity. Based on the t-test at the 5% significance level, the highest antioxidant activity was observed in the leaves of sample A3. The environmental conditions at the sampling sites are characterized by climate type A and are classified as lowland. Samples A1 and A2 originated from Inceptisol soils, while sample B was collected from Ultisol soils. Climatic and weather factors did not significantly influence the results.

5. Acknowledgment

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