Trop J Nat Prod Res, December 2025; 9(12): 5992 - 6002
ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

*Original Research Article*

# Xanthine Oxidase Inhibition and Metabolite Profiling of *Aquilaria malaccensis* Lam Leaves Extract and Fraction Using FTIR-LCMS

Aris Suhardiman[1,2,3]*, Muhaimin[2,4], Anis Y Choerunisa[5], Yani Mulyani[6]

[1]*Doctoral Program in Pharmacy, Faculty of Pharmacy, Padjadjaran University, Sumedang 45363, Indonesia*
[2]*Department of Biological Pharmacy, Faculty of Pharmacy, Padjadjaran University, Sumedang 45363, Indonesia*
[3]*Department of Biological Pharmacy, Faculty of Pharmacy, Bhakti Kencana University, Bandung 40614, Indonesia*
[4]*Center of Herbal Study, Padjadjaran University, Sumedang 45363, Indonesia*
[5]*Department of Pharmaceutical Technology Pharmacy, Faculty of Pharmacy, Padjadjaran University, Sumedang 45363, Indonesia*
[6]*Department of Pharmacology Clinical Pharmacy, Faculty of Pharmacy, Bhakti Kencana University, Bandung 40614, Indonesia*

ARTICLE INFO

*Article history:*
Received 09 September 2025
Revised 13 November 2025
Accepted 04 December 2025
Published online 01 January 2026



ABSTRACT

Clinically, hyperuricaemia is caused by increased uric acid synthesis catalysed by the enzyme xanthine oxidoreductase. This enzyme exists in two forms: xanthine dehydrogenase, which oxidises hypoxanthine to xanthine, and xanthine oxidase, which oxidises xanthine to uric acid. Agarwood leaves (*Aquilaria malaccensis* Lam.) possess antihyperuricemic activity. The flavonoid compounds contained in agarwood leaves are thought to inhibit the production of this enzyme. This study aimed to assess the inhibitory activity of agarwood leaf extracts and fractions against xanthine oxidase and to identify the active compounds in the extracts and fractions. Agarwood leaves were macerated with 96% ethanol and fractionated by liquid-liquid extraction with n-hexane, ethyl acetate, and water. The ethanol extract and fractions obtained were tested for their inhibitory activity against xanthine oxidase in vitro using a spectrophotometer at 295 nm. To determine the functional groups and active compounds, the extracts were analysed by Fourier transform infrared spectroscopy (FTIR), and the ethyl acetate fractions were analysed by liquid chromatography-mass spectrometry (LC-MS) and FTIR. The results showed that the percentage of xanthine oxidase inhibition was 92.05%, 47.88%, 91.17%, and 57.60% for the ethanol extract, n-hexane fraction, ethyl acetate fraction, and aqueous fraction, respectively. FTIR analysis showed the presence of O–H functional groups, while LC-MS analysis showed flavonoids as the main constituents. It can be concluded that the ethyl acetate fraction contains flavonoids that can inhibit the xanthine oxidase enzyme.

***Keyword:*** *Aquilaria malaccensis* L., Ethylacetate Fraction, Flavonoids, Xanthine Oxidase Inhibition

## Introduction

Agarwood (*Aquilaria malaccensis* Lam.) is a native Indonesian species traditionally used by the Dayak community in Central Kalimantan as a medicinal plant, commonly processed into tea. Agarwood leaves have been employed in Southeast Asian traditional medicine as stimulants or sedatives to treat a variety of diseases, including cardiovascular, joint, and diarrheal disorders.[1] Agarwood leaves also have antioxidant, antibacterial, anti-inflammatory and antimutagenic properties,[2] antibacterial,[3] anti-tumour, analgesia, sedation, anti-inflammatory, cough, asthma, antidepressant,[4] antioxidant, antiaging, and cardiovascular system.[1] The pharmacological activities of agarwood leaves are attributed to secondary metabolite content. As a result, medicinal plants are seen as a potential source of new therapeutic molecules, especially for oxidative stress-related degenerative disorders.[5] According to its definition, oxidative stress is a pathological state marked by an overabundance of reactive oxygen species (ROS), Such as superoxide radicals, hydroxyl radicals, and lipid peroxides. Under normal conditions, these radicals can be neutralised by endogenous or natural antioxidants. However, when the balance between free radical production and antioxidant defence is disrupted, excessive ROS accumulation occurs, leading to oxidative damage and the progression of various degenerative diseases, including hyperuricemia.[6] Oxidative stress and the resulting cellular damage influence cell behaviour, proliferation, adaptation, injury response, ageing, and may induce apoptosis or necrosis.[7]

Degenerative diseases, such as hyperuricemia resulting from excess free radicals, can be prevented or improved with secondary metabolites, especially flavonoids. Agarwood is known to contain flavonoids, which contribute to its antihyperuricemic activity.[8] The antioxidant and antihyperuricemic effects of flavonoids are closely related to their structural characteristics, particularly the number and position of hydroxyl groups. Elevated blood uric acid levels are a hallmark of hyperuricemia, a clinical symptom of gout. One of the primary causes of this disease is the breakdown of purines by the enzyme xanthine oxidase (XO), which catalyses the conversion of hypoxanthine to xanthine and then to uric acid. Therefore, the antihyperuricemic potential of a compound can be evaluated through the ability to inhibit xanthine oxidase activity.[9] Previous studies on *Aquilaria crassna* showed significant antihyperuricemic activity both *in vitro* and *in vivo*. Extract showed xanthine oxidase inhibitory activity with an $IC_{50}$ value of 1.35 ± 0.03 mg/mL. Furthermore, oral administration at concentrations of 1000 and 3000 mg/kg reduced uric acid production by 47.2% and 63.6%, respectively.[10] The results provide scientific

*Corresponding author. Email: aris22002@mail.unpad.ac.id
Tel: +62 85211123403

Citation. Suhardiman A, Muhaimin , Choerunisa Y A, Mulyani Y. Xanthine Oxidase Inhibition and Metabolite Profiling of *Aquilaria malaccensis* Lam Leaves Extract and Fraction Using FTIR-LCMS. Trop J Nat Prod Res. 2025; 9(12): 5992 – 6002 https://doi.org/10.26538/tjnpr/v9i12.12

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

evidence supporting the potential of Agarwood leaves as a natural source of antihyperuricemic agents.

Based on the above background, the objectives of this study are to determine the xanthine oxidase inhibitory activity of agarwood leaf extracts and fractions, and to identify the ethyl acetate fraction as the active fraction using LC-MS. An innovative analytical technique called Liquid Chromatography-Mass Spectrometry (LC-MS) combines the molecular identification potential of mass spectrometry with the separation power of liquid chromatography. This method first separates complex mixtures in the liquid chromatography system by utilising interactions between the stationary and mobile phases. Mass-to-charge (m/z) ratios are used to detect separated chemicals in the mass spectrometer, yielding mass spectra that reveal the sample's molecular makeup.[11] Fourier Transform Infrared Spectroscopy (FTIR) is another analytical tool used to characterise chemical constituents through infrared absorption spectra. This approach is consistent with the idea that molecular bonds absorb infrared light at particular frequencies that correlate to vibrational energy. Chemical structures can be identified thanks to the unique absorption patterns of each functional group. Fourier transform mathematically converts raw time-domain signals into frequency-domain spectra. This results in infrared spectra with characteristic absorption bands that reveal the functional groups present in the sample.[12]

This study evaluates the *in vitro* antihyperuricemic activity of agarwood leaf extract and three fractions using xanthine oxidase inhibition, and identifies the active compounds in the most potent fraction using FTIR and LC-MS. This study highlights the unexplored potential of agarwood leaf extract and fractions as xanthine oxidase inhibitors, along with their metabolite profiling.

## Materials and Methods

*Reagents and Chemicals*

The chemicals used were Xanthinoxidase enzyme and substrate (Sigma-Aldrich), 95% ethanol (Brataco), n-hexane (Brataco), ethyl acetate (Brataco), distilled water (Brataco), Quercetin (Markherb), and Gallic Acid (Markherb). All other chemicals and reagents were of analytical grade.

*Plant Collection and Identification*

In this study, dried agarwood leaves were collected on 4 January 2023 by PT Mitra Dulur Sejahtera at Jalan Bala Dewa No. 8, Bukit Lama, Ilir Barat I District, Palembang City, South Sumatra 30121, with coordinates -2.9925349356067734, 104.72987561487965. The sample was taxonomically identified at the School of Life Sciences and Technology – ITB, Labtek XI, Jalan Ganeca No. 10, Lb. Siliwangi, Coblong District, Bandung City, West Java, Indonesia, where a voucher specimen with number 982/IT1.C11.2/TA.00/2023 was deposited. Dried agarwood leaves were stored in a tightly sealed container in a locker in the pharmacognosy laboratory at Bhakti Kencana University.

*Simplicia Characterisation*

Dried agarwood leaves (*Aquilaria malaccensis* Lam) were tested for their characteristics, including organoleptic analysis, determination of drying shrinkage, total ash content, acid-insoluble ash content, water-soluble extract content, ethanol-soluble extract content, and moisture content. [13]

*Organoleptic examination*

Agarwood leaf simplicia was evaluated organoleptically by observing shape, colour, taste, and odour.[13]

*Determination of drying shrinkage*

Approximately 2 g of Agarwood leaves simplicia were weighed using a moisture balance and dried at 105 °C until a constant weight, starting from the activation of the heating lamp until it automatically turned off. The results were recorded directly from the instrument display.[13]

*Determination of total ash content*

Agarwood leaf powder (2 g) was weighed, put in a crucible, and heated until its weight remained constant. The sample was charred on a hot plate until and then further ashed in a furnace. The residue was cooled in a desiccator and then weighed. When there was still charcoal, hot water was added, and the mixture was filtered through ash-free filter paper. After that, the residue and filter paper were burned in a crucible, and the process was repeated until the weight was constant.[13]

*Determination of acid-insoluble ash content*

To determine which ash was acid-insoluble, the entire quantity of ash was heated for five minutes after being treated with 25 mL of dilute hydrochloric acid. The mixture was run through ash-free filter paper, and the crucible was then rinsed with hot water. The residue was collected, then transferred to the crucible and heated in a furnace until its weight remained constant. After cooling in a desiccator, the final weight of the acid-insoluble ash was recorded.[8]

*Determination of water-soluble essence content*

After weighing about 5 g of powdered Agarwood leaves into a stoppered flask, 100 mL of water saturated with chloroform was added. After vigorous shaking for the first 6 hours, the flask was left to stand for 18 hours. After filtering the mixture, 20 mL of the filtrate was dried in a porcelain dish by evaporation. After drying the residue to a constant weight at 105°C, the water-soluble extractive value was determined.[8]

*Determination of ethanol-soluble juice content*

About 5 g of powdered agarwood leaves were weighed into a stoppered flask and extracted using 100 mL of 96% ethanol. The flask was shaken often for six hours before being left to stand for eighteen hours. Following the extraction process, 20 mL of the filtrate was evaporatively dried on a porcelain plate. After drying the residue at 105°C to constant weight, the ethanol-soluble extractive value was determined.[8]

*Determination of moisture content*

Agarwood leaves simplicia (2 g) were added to a flask that held 2 mL of distilled water and 200 mL of toluene. It was left to stand for 2 hours, and the flask was allowed to cool to ambient temperature. A rubber stopper attached to a copper wire soaked in water-saturated toluene was used to dislodge water droplets that had stuck to the condenser tube until all had fallen into the graded tube. After complete separation from toluene, the water volume was measured to determine the moisture content.[8]

*Extract and fractionation of plant materials*

Using a solvent-to-sample ratio of 10:1, dried agarwood leaves were ground and macerated with 96% ethanol for three to twenty-four hours at room temperature. The solvent was renewed every 24 hours, and the combined liquid extract was collected in tightly sealed containers. A rotary evaporator was used to filter and concentrate the extract, resulting in a viscous form, and the yield was computed.[8] Then, using n-hexane, ethyl acetate, and water as solvents, 20 g of concentrated ethanol extract was separated by liquid–liquid extraction. The procedure was repeated until the n-hexane, ethyl acetate, and aqueous fractions were obtained. Each fraction was concentrated to dryness using a rotary evaporator, and the yield of each fraction was calculated.[14,8,15]

*TLC Profile of Extract and Fraction*

In this study, TLC profiles of agarwood leaf extract and fraction were analysed using silica gel TLC plates. Different solvent systems were used for the elution of the plates, including n-hexane: ethyl acetate (8:2), chloroform: methanol (8:2), and ethyl acetate:methanol: distilled water (8:1:1). Detection was carried out using $FeCl_3$ 10% in methanol, $H_2SO_4$ 10% in methanol, $AlCl_3$ 5% in methanol, and DPPH 0.2% in methanol as spraying reagents. The chromatograms were visualised under UV light at 254 nm and 366 nm.[8]

*Phytochemical Screening*

Phytochemical screening of the simplicia and agarwood leaves extract was performed using specific reagents to detect the presence of secondary metabolites.[8]

*Determination of Total Flavonoid Content*

Using quercetin as the reference, UV-Vis spectrophotometry was used to measure the total flavonoid concentration at 420 nm. In this assay, the agarwood leaf extract was dissolved in ethanol, and 0.5 mL of the mixture was transferred into a 1.5 mL tube. 2.8 mL of distilled water, 0.1 mL of a 10% $AlCl_3$ solution, and 0.1 mL of a 1 M potassium acetate

solution were added. The mixture was incubated at room temperature for 30 minutes. Quercetin at different concentrations was used to create a calibration curve, and absorbance was measured between 400 and 450 nm. The total flavonoid content was calculated from the calibration curve and expressed as quercetin equivalents per 100 mg of extract (mg QE/100 mg).[16]

*Determination of Total Phenolic Content*

Gallic acid was used as the standard in the Folin–Ciocalteu technique to calculate the total phenolic content. All measurements were made in triplicate, with methanol serving as the blank. For the experiment, 1.58 mL of distilled water, 100 µL of Folin–Ciocalteu reagent, and 300 µL of sodium carbonate solution (200 g/L) were combined with 20 µL of gallic acid standard or sample solution. After vortexing, the mixture was incubated in a water bath at 40°C for 30 minutes. Subsequently, absorbance was measured at 600–750 nm using a UV-Vis spectrophotometer.[16] The result of the total phenolic content was calculated from the calibration curve and expressed as gallic equivalents per 100 mg of extract (mg GAE/100 mg).

*Xanthine Oxidase Enzyme Inhibition Assay*

The test was performed according to the modified Sigma-Aldrich procedure at a detection wavelength of 550 nm. The test samples (100 mg) were dissolved in 0.5% DMSO with $CO_2$-free water to get a final volume of 100 mL. After adding 2 µL of fluorescent peroxidase substrate to each microplate well, the reaction mixture contained 50 µL of the sample solution, 44 µL of xanthine oxidase assay buffer, 2 µL of mixed substrate, and 2 µL of xanthine oxidase enzyme. The ethanol extract and fractions (n-hexane, ethyl acetate, and aqueous) from Agarwood were tested at various concentrations, as shown in Table 2, with allopurinol serving as the positive control. The percentage inhibition of xanthine oxidase was calculated for all test samples and compared with allopurinol.[16]

*Compound Identification by LC-MS/MS*

Approximately 1 mg of the ethyl acetate fraction was dissolved in ethanol, and 20 µL of the solution was injected into an LC-MS/MS system (Waters, USA) equipped with an electrospray ionisation (ESI) source operating in positive ion mode and a time-of-flight (TOF) detector. Separation was achieved using a C18 column with a methanol: water (8:2) mobile phase. The chromatograms and mass spectra were analysed using MassLynx software (version 4.1), and compound identification was performed by comparing the base peak data with reference spectra from the ChemSpider and HMDB databases.[17]

*Compound Identification by FTIR*

The functional groups of the ethyl acetate fraction were identified using the Agilent Cary 630 FTIR spectrometer. The instrument was calibrated before analysis, and the sample was prepared as a thin film between NaCl or KBr plates. Spectra were recorded over 4000–400 $cm^{-1}$ with adequate resolution and signal quality. The absorption bands were analysed and compared with reference FTIR spectral libraries to determine the functional groups present. Each peak corresponds to the characteristic vibration of a specific chemical bond; values represent mean ± SD, n=3.

## Results and Discussion

The organoleptic evaluation of Agarwood leaves simplicia showed that the leaves were oval in shape, tapered at the tips, green in colour, possessed a distinctive odour, and had a bitter taste. Physicochemical characterisation showed that the simplicia had a drying shrinkage of 6.25%, a moisture content of 4.97%, a water-soluble extractive value of 0.62%, an ethanol-soluble extractive value of 10.29%, a total ash content of 5.67%, and an acid-insoluble ash content of 0.49%. The moisture content of simplicia should not exceed 10%, as levels above this may increase the risk of microbial and fungal growth. The moisture content of Agarwood leaves simplicia (4.97%) was well below this limit, showing good stability and storability. The total ash value (5.67%) showed the total inorganic content and falls within the acceptable range for herbal raw materials, suggesting low contamination by extraneous matter. The acid-insoluble ash value (0.49%) was also within the permissible limit (<1%), showing minimal silica or sand contamination.[18] The extractive values indicate different classes of phytoconstituents. The water-soluble extractive value (10.62%) and ethanol-soluble extractive value (10.29%) suggest that agarwood leaves contain both polar and moderately non-polar secondary metabolites, such as flavonoids, tannins, saponins, and terpenoids. Similar results had been reported in previous studies, where agarwood leaves were shown to contain flavonoids and phenolic compounds with significant antioxidant and anti-inflammatory activities.[19] The organoleptic and physicochemical parameters obtained in this study show that Agarwood leaves simplicia meet the required quality standards for use as raw material in herbal formulations, while also suggesting the presence of bioactive compounds contributing to the pharmacological potential.

The simplicia was extracted with 96% ethanol by maceration due to its simplicity, usefulness, and the absence of heating, which prevents thermolabile chemicals from degrading. During maceration, the solvent penetrated the cell walls and intracellular spaces of the plant material, allowing the secondary metabolites to dissolve into the Solvent.[18] Ethanol 96% showed a polarity index of 5.2, which enabled the extraction of broad spectra of secondary metabolites, including polar, semi-polar, and non-polar Compounds.[18] The extraction process using the maceration method of agarwood leaves produced an ethanol extract yield of 13.23%. Extract yield is one of the parameters used to determine whether bioactive compounds in agarwood leaf extracts are soluble or insoluble in solvents based on their polarity, thereby indicating whether they are polar, semi-polar, or non-polar.[18] Fractionation was conducted to obtain compounds of higher purity by using solvents with different polarities. This method is based on the principle of *"like dissolves like"*, stating that polar compounds are more soluble in polar solvents. In contrast, non-polar compounds dissolve better in non-polar solvents.[14] In this study, three solvents of varying polarity were used, namely n-hexane, ethyl acetate, and water. The fractionation process produced yields of 11.10%, 41.82%, and 47.08% for n-hexane, ethyl acetate, and water, respectively. The yield of the aqueous fraction suggested that the ethanol extract of agarwood leaves contained a higher proportion of polar compounds compared to semi-polar and non-polar constituents.[20]

The chemical profiling of agarwood extract was conducted using TLC on silica gel $GF_{254}$ plates, using non-polar, semi-polar, and polar mobile phases to optimise separation and identify constituent compounds. Phenolic compounds were detected by the appearance of black spots upon spraying with 10% $FeCl_3$. In comparison, the presence of flavonoids was confirmed by blue fluorescence under UV light at 366 nm after treatment with 5% $AlCl_3$.[21,22] Antioxidant activity was evaluated using a post-chromatography DPPH assay. Spots showing antioxidant behaviour turned yellow against a purple background after DPPH treatment, showing free radical scavenging activity, when chromatographed using semi-polar and polar mobile phases. There was no colour change with non-polar eluents, suggesting weaker antioxidant activity in the non-polar fraction. This pattern is consistent with the TLC-DPPH method, in which antioxidants are visualised as yellow bands, indicating active phytochemical constituents.[20,23] The elution of the TLC plate using semi-polar and polar solvents led to the demonstration of antioxidant activity in the extract and fraction. This observation was characterised by a colour change in the sample, which turned yellow after exposure to DPPH. In the case of non-polar eluents, there was no colour change due to the comparatively weak antioxidant activity shown by non-polar compounds. The outcomes of the TLC monitoring of the extract and fraction are shown in Figure 1.

The simplicia and ethanol extract of agarwood leaves were confirmed to contain flavonoids, which were in line with the results from the TLC profile analysis. Flavonoids, a class of polyphenolic secondary metabolites, are widely known for their antioxidant capabilities, which function through multiple mechanisms. Furthermore, flavonoids can inhibit pro-oxidant enzymes, such as xanthine oxidase, NADPH oxidase, and lipoxygenase, thereby decreasing ROS generation. These metabolites can strengthen cellular defences against oxidative stress by upregulating endogenous antioxidant enzymes, such as glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD).

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

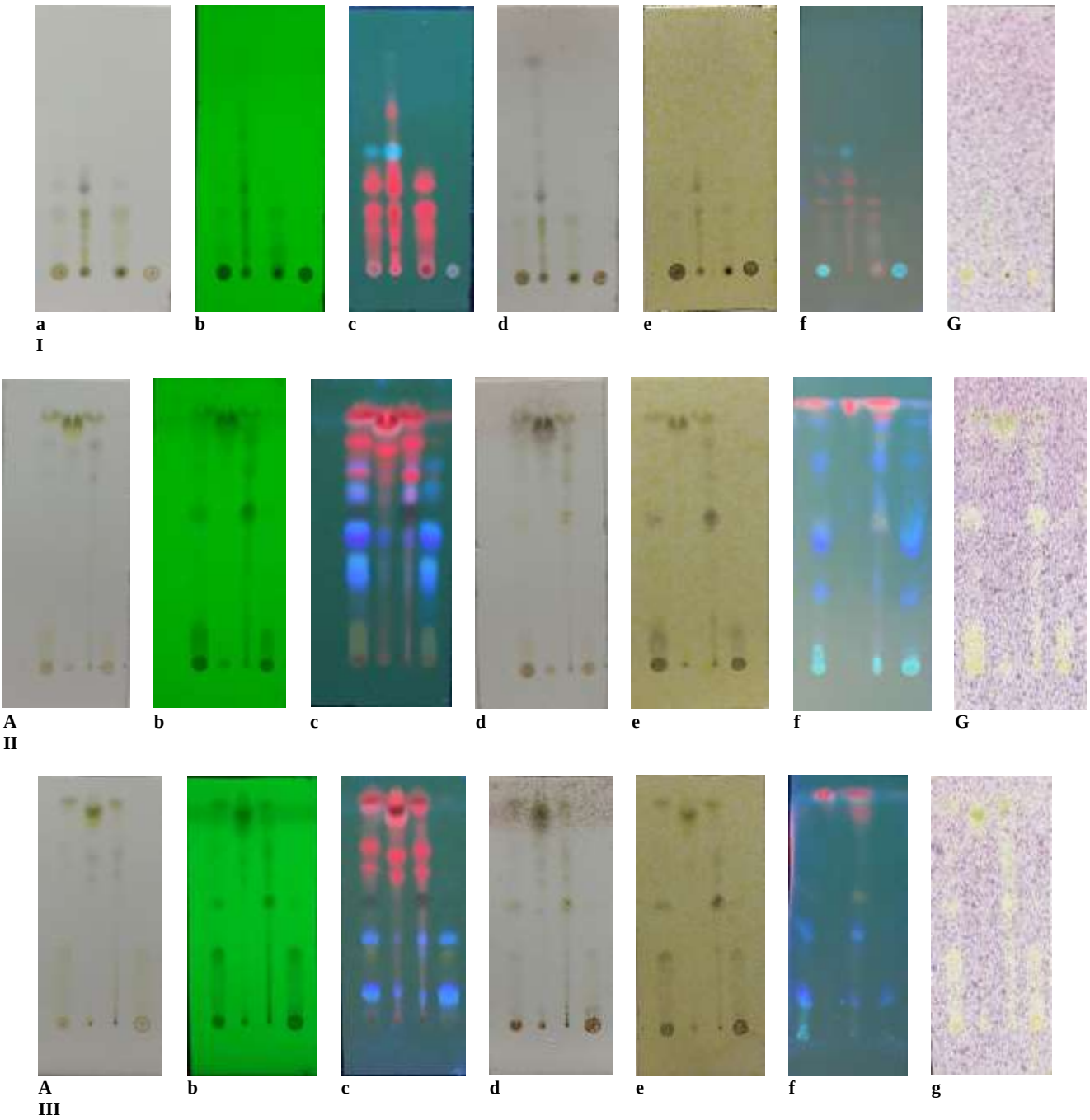



**Figure 1:** Chromatogram of Agarwood Leaf Extract and Fraction

Image caption: The chromatogram of the extract using silica gel GF254 stationary phase with developers n-hexane: ethyl acetate (8:2) (I), chloroform: methanol (8:2) (II) and ethyl acetate:methanol: distilled water (8:1:1) (III); agarwood leaf extract (1), N-hexane fraction (2), ethyl acetate fraction (3), ethanol-Aqueous fraction (4); Visual observation (a), UV λ 254 nm (b), UV λ 365 nm (c), visual spotting of 10% $H_2SO_4$ (d), spotting of 10% $FeCl_3$ (e), spotting of 5% $AlCl_3$ under UV light λ 365 nm (f), spotting of DPPH (g).

The results support the potential of Agarwood leaves simplicia and extract as a natural source of antioxidants capable of mitigating oxidative damage.[24,25] Utilising TLC with several mobile phases, the presence of flavonoids and phenolics in agarwood leaves was verified. Visualisation under UV light at 254 nm produced fluorescent plates with dark spots, whereas at 366 nm, the spots fluoresced against a dark background. This showed the interaction between UV light and the chromophore groups in compounds. TLC plates developed with chloroform: methanol (8:2) and ethyl acetate:methanol: water (8:1:1) under UV at 366 nm showed parallel spots, consistent with the presence of flavonoids. Spotting with $FeCl_3$, $AlCl_3$, and $H_2SO_4$ produced similar patterns indicative of phenolic and flavonoid constituents. These observations suggest the presence of antioxidant-active compounds in agarwood leaves, as flavonoids and phenolics are known for their radical-scavenging properties.[22&23]

The phytochemical screening aimed to identify the classes of secondary metabolites present in the simplicia and ethanol extracts of agarwood leaves. Alkaloids, flavonoids, tannins, saponins, quinones, and steroids/triterpenoids were all detected using qualitative assays. Flavonoids, saponins, tannins, quinones, steroids/triterpenoids, and alkaloids were found in simplicia, according to screening results. In comparison, ethanol extract was positive for flavonoids, tannins, quinones, steroids/triterpenoids, and alkaloids, but negative for

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

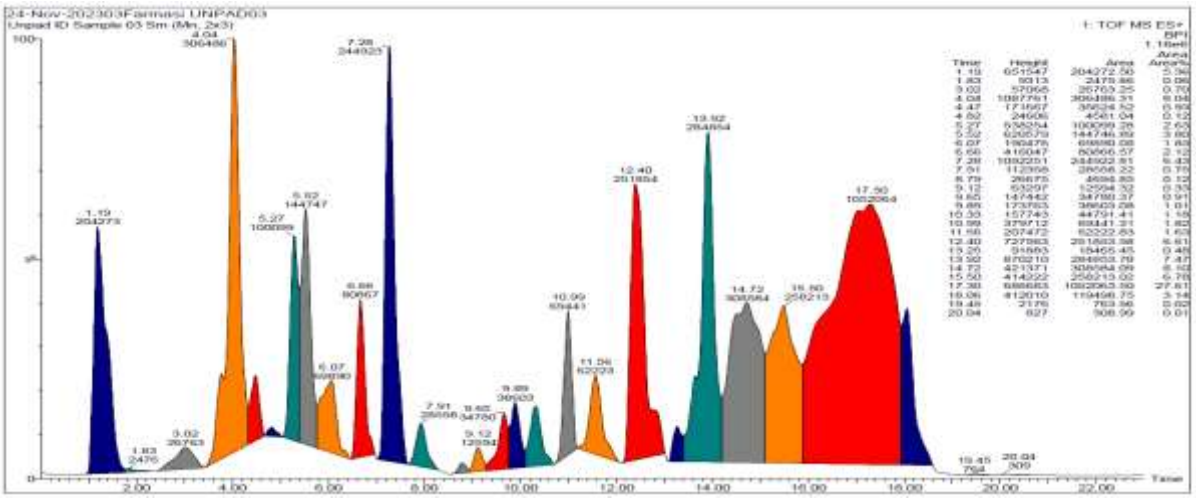


**Figure 2:** LC-MS Chromatogram Profile of Ethylacetate Fraction of Agarwood Leaf (*Aquilaria malaccensis* Lam)

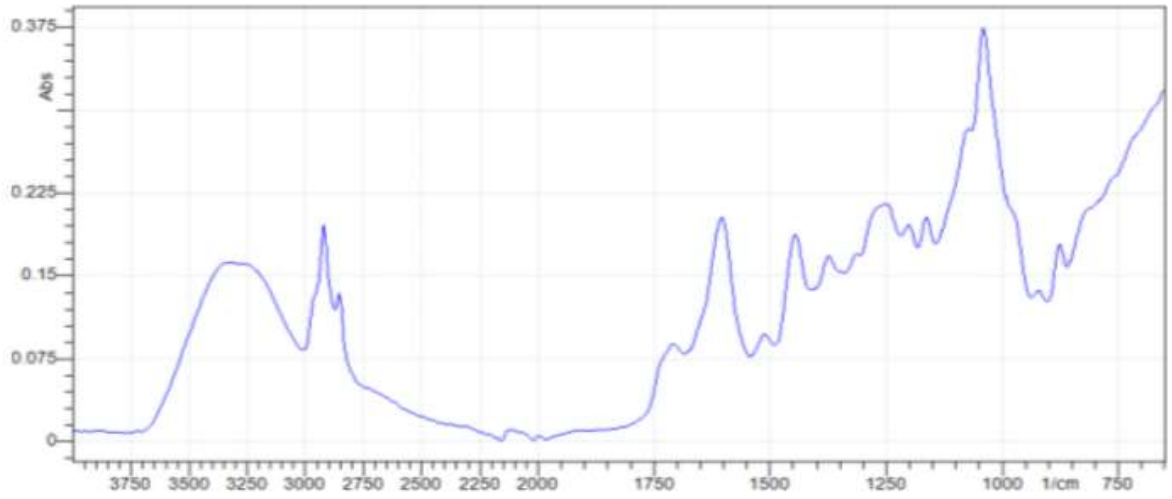



**Figure 3:** FTIR Spectrum of Ethanol Extract of Agarwood Leaf

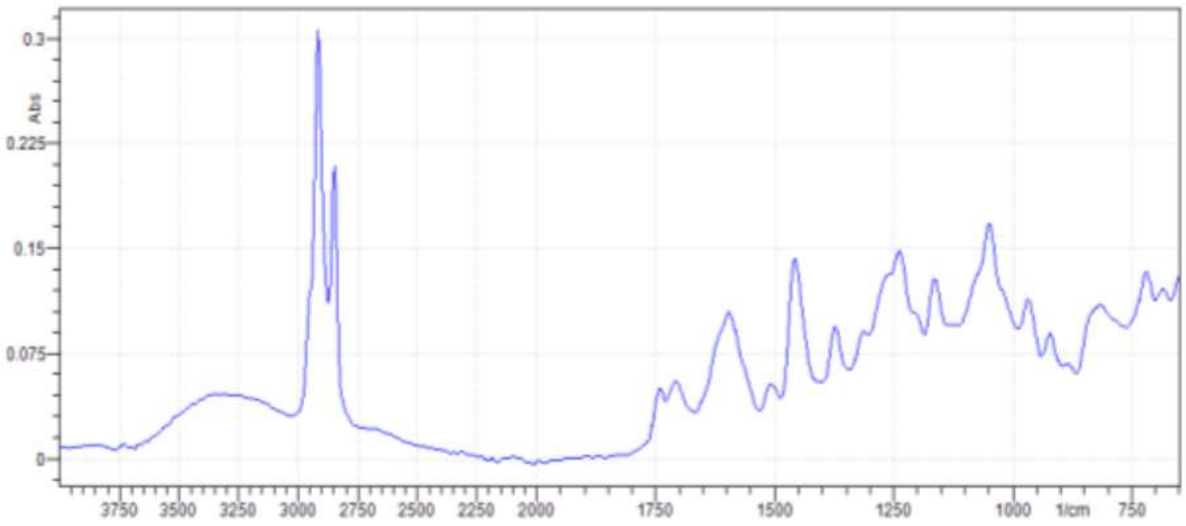
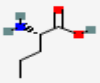
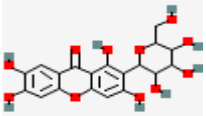
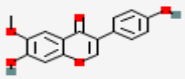
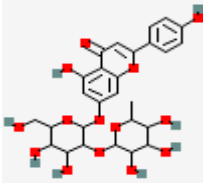
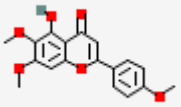
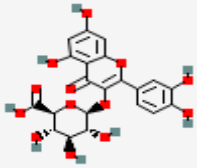
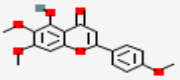
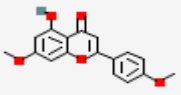
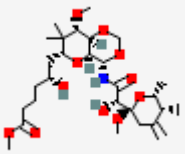



**Figure 4:** FTIR Spectrum of Ethylacetate Fraction of Agarwood Leaf

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

**Table 1:** Compound Analysis Result of LC-MS Chromatogram Profile

| No Peak | Retention Time | % Area | Measured ion Mass | Compound | Group | Reference | Structure (Chemdraw) |
|---|---|---|---|---|---|---|---|
| 1 | 1.19 | 5.36 | 118.0850 | Norvaline | d-alpha-amino acids (isomeric with valine) | ChemSpider ID58608 HMDB0013716 |  |
| 2 | 4.04 | 5.22 | 423.0923 | Mangiferin; 1,3,6,7-tetrahydroxyxanthone C2-*β*-D-glucoside | xanthones | ChemSpider ID4444966 HMDB0034410 |  |
| 3 | 5.27 | 1.69 | 285.0779 | Glycitein; 7,4'-Dihydroxy-6-methoxyisoflavone | isoflavones | ChemSpider ID4476508 HMDB0005781 |  |
| 4 | 5.52 | 1.69 | 579.1734 | Rhoifolin; Apigenin 7-O-neo-hesperidoside | flavonoid-7-o-glycosides | ChemSpider ID4445347; HMDB0038848 |  |
| 5 | 6.68 | 1.36 | 300.266 | Diosmetin: 5,7,3'-trihydroxy-4'-methoxyflavone | flavonoids | ChemSpider ID4444931; HMDB0128577 |  |
| 6 | 7.28 | 86,28 | 479 | Quercetin 3-O-glucuronide; (Miquelianin) | flavonoid-3-o-glucuronides | ChemSpider ID4444920; HMDB0132454 |  |
| 7 | 10.99 | 0.78 | 329.1040 | Salvigenin; 5-Hydroxy-4',6,7-trimethoxyflavone | flavonoids | ChemSpider ID141666; HMDB0128577 |  |
| 8 | 11.56 | 2.75 | 299.0929 | Apigenin 7,4'-dimethyl ether; 5-Hydroxy-4',7-dimethoxy-flavone | flavonoids. | ChemSpider ID4444920; HMDB0132454 |  |
| 9 | 12.40 | 1.78 | 574.3260 | theopederin B | Esters | ChemSpider ID8432983; |  |
| 10 | 13.92 | 0.88 | 440.4100 | 1-Hydroxy-2,2,6,6-tetramethyl-4-piperidinyl stearate | Lipid | ChemSpider ID504303 |  |

saponins. These findings are consistent with earlier research that found saponins in simplicia and ethanol extract from agarwood leaves. Moreover, qualitative phytochemical screening remains a fundamental and cost-effective method for initial identification of key metabolite groups in botanical studies.[20]

The $AlCl_3$ colourimetric method with visible spectrophotometry at 420 nm was used to quantify total flavonoid content using quercetin as the standard reference. A keto group at position C-4 and hydroxyl groups at positions C-3 or C-5, which are structural characteristics of flavones and flavonols, are present in quercetin, a typical flavonoid. A calibration curve was constructed using quercetin standard solutions at concentrations of 50, 60, 70, 80, 90, 100, 110, and 120 ppm. The resulting regression equation was $y = 0.008x + 0.1442$ with a high correlation coefficient ($R^2 = 0.9992$), confirming excellent linearity. Based on this curve, the flavonoid content of the extract was quantified **as** 21.295 ± 0.112 mg QE/g extract. This method is widely adopted for flavonoid determination due to sensitivity and reproducibility in detecting flavones and flavonols.[26]

Gallic acid was used as the standard reference compound in the Folin–Ciocalteu colourimetric technique to evaluate the total phenolic content. A calibration curve was constructed using gallic acid solutions at concentrations of 20, 40, 60, 80, 100, and 120 ppm. The linear regression equation obtained was $y = 0.0062x + 0.0723$, with a correlation coefficient ($R^2$) of 0.9943, indicating strong linearity between concentration (x) and absorbance (y). Based on this calibration curve, the total phenolic content of agarwood leaf extract was quantified as 11.254 ± 0.23 g GAE/100 g extract**.**[26],[27] The bioactive substances in the ethanol extract of agarwood leaves (total flavonoid and total phenolic content) were quantitatively determined in this study. Flavonoids and phenolics exhibit antioxidant activity by scavenging free radicals, chelating metals, and modulating cellular antioxidant enzymes. Additionally, several studies have shown potential antihyperuricaemic activity by inhibiting xanthine oxidase, a key enzyme in uric acid biosynthesis.[28] Research on *Dennetia tripetala* roots shows experimental evidence of robust antioxidant activity. This can be attributed to the high levels of total phenolics as antioxidant compounds found in the roots. These findings are considered essential, as they may provide a biochemical basis for several medicinal uses of *Dennetia tripetala* roots.[29]

The inhibitory activity of Agarwood leaves against xanthine oxidase enzyme was evaluated using ethanol extract, n-hexane, ethyl acetate, and aqueous fraction. Based on the results, the ethyl acetate fraction showed the strongest xanthine oxidase inhibition (54.06%) at 10 µg/mL. Ethanol extract and the aqueous fraction also showed significant inhibitory effects, with 51.41% and 50.53% inhibition, respectively, at concentrations of 125 µg/mL. Meanwhile, the n-hexane fraction showed the lowest activity, with 47.88% inhibition at 200 µg/mL. The findings indicate that agarwood leaf ethanol extract and the ethyl acetate fraction both exhibit strong xanthine oxidase inhibitory activity, on par with allopurinol, a common xanthine oxidase inhibitor used in clinical settings to treat hyperuricaemia. The ethyl acetate fraction in particular shows the most significant promise. Since flavonoids and phenolic compounds have been shown in multiple studies to inhibit xanthine oxidase by chelating the enzyme's molybdenum centre and reducing superoxide production during purine catabolism, the observed activity may be related to their presence.[30]

In this study, agarwood leaves were extracted into four fractions (ethanol, n-hexane, ethyl acetate, and water), and the inhibitory effects on xanthine oxidase were evaluated. The results of the xanthine oxidase inhibition assay are presented in Table 2. The ethyl acetate fraction showed the most potent inhibitory activity, achieving 54.06% ± 2.50 inhibition at 10 µg/mL. In comparison, the ethanol extract, n-hexane, and aqueous fraction reached approximately 50% inhibition only at higher concentrations. The positive control, allopurinol, exhibited 50% inhibition at a much lower concentration (2.75 µg/mL). These results showed that both the ethanol extract and the ethyl acetate fraction of agarwood leaves exhibit significant xanthine oxidase inhibitory activity, with the ethyl acetate fraction showing the most potent activity. The inhibitory effect observed suggests a mechanism comparable to that of allopurinol, a standard clinical treatment for hyperuricaemia. By inhibiting xanthine oxidase, this fraction can reduce uric acid production and mitigate oxidative stress associated with hyperuricaemia. The activity is consistent with previous reports showing that flavonoids and phenolic compounds act as natural inhibitors because of the ability to chelate molybdenum at the active site of the enzyme and scavenge ROS.[31, 32]

Increased uric acid levels cause oxidative stress, which has been linked to several degenerative illnesses, such as kidney disease, cardiovascular disease, diabetes, and hypertension. The process by which xanthine oxidase catalyses the reduction of molecular oxygen to superoxide anion and subsequent conversion to hydrogen peroxide is intimately linked to the pathophysiology. Excess hydrogen peroxide, as a ROS, can exert cytotoxic effects and contribute to tissue damage**.**[33] Therefore, serum uric acid levels should not exceed 6.8 mg/dL to prevent pathological complications.[34,35] Based on the pathogenesis of gout, two main therapeutic strategies are used, namely inhibition of inflammation and reduction of serum uric acid levels. The management of acute gouty arthritis commonly includes colchicine (COL), corticosteroids, and non-steroidal anti-inflammatory drugs (NSAIDs). In comparison, extracts need much higher concentrations, and long-term management to reduce serum uric acid levels primarily depends on xanthine oxidase inhibitors, such as allopurinol and febuxostat.[36,37,35]

The results of testing ethanol extracts, ethyl acetate fractions, aqueous fractions, and hexane fractions from agarwood leaves (*Aquilaria malaccensis* Lam) for xanthine oxidase enzyme inhibition activity were analysed using a one-way ANOVA method. One-way ANOVA is the first step to ensure that the data to be tested meet the assumption of normality, namely that each data group comes from a normally distributed population. The ANOVA test begins with a normality test. This test uses one-way ANOVA, a parametric test that assumes normality, followed by a homogeneity test. The homogeneity test in a one-way ANOVA analysis is used to ensure that the variances across data groups are equal.[38] The normality test results for a one-way ANOVA of agarwood leaf extracts and fractions indicated normally distributed data. The normality test results are shown in Table 3. Meanwhile, the results of the homogeneity test for agarwood leaf extracts and fractions indicate that the variances across the data groups are equal. The results of the homogeneity test for ethanol extract, hexane fraction, ethyl acetate fraction, and aqueous fraction were not significant ($p > 0.05$). Thus, the homogeneity test in one-way ANOVA plays an essential role in ensuring equal data distributions across groups, thereby ensuring that the results of the analysis of mean differences are objective and statistically valid.[38] The results of the homogeneity test are presented in Table 4. The analysis of variance between five independent groups (ethanol extract, hexane, ethyl acetate, and aqueous fractions) compared with allopurinol, a drug with a xanthine oxidase inhibition mechanism, shows a p-value < 0.05, indicating a difference between groups. Research conducted to test the inhibitory activity of xanthine oxidase enzymes from ethanol extracts, hexane fractions, ethyl acetate fractions, and aqueous fractions, compared with allopurinol, showed significant differences in the percentage of inhibition. This condition ensures that the test results are valid, reliable, and representative of the population being compared.

The active fraction of ethyl acetate in agarwood leaves was analysed by LC-MS using MassLynx 4.1 software. LC-MS is a method used to identify and predict compounds based on mass values. In the chromatogram, each peak corresponds to a specific compound. Using Masslynx 4.1, the software selects the compound with the highest fit confidence score, which measures how well the molecular formula matches the detected compound. In this study, 20 peaks were analysed, and 10 showed fit conf (%) values of over 70%. These compounds were identified as the active fraction with the highest xanthine oxidase enzyme inhibition activity, as shown in Figure 2. From the results of the LC-MS chromatogram profile of the ethylacetate fraction, 10 peaks were analysed for compound identification. The ethylacetate fraction obtained from agarwood leaves (*Aquilaria malaccensis* Lam.) was found to contain a variety of flavonoids, including glycitein, rhiofolin, diosmetin, quercetin, savigenin, and apigenin, as well as xanthones such as mangiferin. The presence of these compounds suggested that agarwood leaves may have the potential to inhibit xanthine oxidase, indicating their suitability for use in antihyperuricaemia herbal therapy. The identification of flavonoids and phenolic compounds in agarwood

**ISSN 2616-0684 (Print)**
**ISSN 2616-0692 (Electronic)**

**Table 2:** Results of the xanthine oxidase enzyme inhibition assay

| No | Sample | Concentration (µg/ml) | Average % Inhibition | Std. Dev |
|---|---|---|---|---|
| 1 | Allopurinol (Control +) | 1.75 | 16.96 | 2.50 |
| | | 2 | 24.91 | 1.25 |
| | | 2.25 | 39.05 | 1.25 |
| | | 2.75 | 44.35 | 2.50 |
| 2 | Ethanol Extract | 75 | 39.93 | 2.50 |
| | | 100 | 44.35 | 1.25 |
| | | 125 | 51.41 | 3.75 |
| | | 150 | 54.06 | 2.50 |
| | | 175 | 62.01 | 1.25 |
| | | 200 | 67.31 | 3.75 |
| | | 225 | 76.15 | 1.25 |
| | | 250 | 80.57 | 2.50 |
| | | 275 | 88.52 | 1.25 |
| | | 300 | 92.05 | 1.25 |
| 3 | Ethylacetate fraction | 5 | 49.65 | 1.25 |
| | | 1 | 54.06 | 2.50 |
| | | 25 | 60.25 | 1.25 |
| | | 50 | 65.55 | 3.75 |
| | | 75 | 72.61 | 1.25 |
| | | 100 | 77.03 | 2.50 |
| | | 125 | 82.33 | 2.50 |
| | | 150 | 84.10 | 2.50 |
| | | 175 | 88.52 | 1.25 |
| | | 200 | 91.17 | 2.50 |
| 4 | Aqueous fraction | 5 | 40.81 | 1.25 |
| | | 10 | 41.70 | 2.50 |
| | | 25 | 42.58 | 1.25 |
| | | 50 | 44.35 | 1.25 |
| | | 75 | 46.11 | 1.25 |
| | | 100 | 47.88 | 1.25 |
| | | 125 | 50.53 | 2.50 |
| | | 150 | 53.18 | 3.75 |
| | | 175 | 54.95 | 3.75 |
| | | 200 | 57.60 | 2.50 |
| 5 | hexane fraction | 50 | 11.98 | 1.25 |
| | | 75 | 35.51 | 1.25 |
| | | 100 | 39.05 | 1.25 |
| | | 125 | 40.81 | 1.25 |
| | | 150 | 43.46 | 0.00 |
| | | 175 | 46.11 | 1.25 |
| | | 200 | 47.88 | 1.25 |

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

**Table 3:** Results of one-way ANOVA analysis: Test of Normality

| | | Kolmogorov-Smirnov[a] | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|---|
| | Sample | Statistic | df | Sig. | Statistic | df | Sig. |
| Score | Allopuriol | .230 | 4 | . | .940 | 4 | .657 |
| | Ethanol Extract | .136 | 10 | .200* | .951 | 10 | .683 |
| | Ethylacetate fraction | .165 | 10 | .200* | .886 | 10 | .153 |
| | Aqueous Fraction | .338 | 10 | 0.002 | .734 | 10 | .002 |
| | Hexane fraction | .281 | 7 | 0.099 | .776 | 7 | .023 |

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

**Table 4:** Test of Homogeneity of Variances

| | | Levene Statistic | df1 | df2 | Sig. |
|---|---|---|---|---|---|
| Score | Based on Mean | .845 | 4 | 36 | .506 |
| | Based on Median | .793 | 4 | 36 | .537 |
| | Based on Median and with adjusted df | .793 | 4 | 27.105 | .540 |
| | Based on the trimmed mean | .839 | 4 | 36 | .509 |

**Table 5:** One-Way ANOVA analysis

**ANOVA**

Score

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 82861898.549 | 4 | 20715474.637 | 5.833 | .001 |
| Within Groups | 127853899.207 | 36 | 3551497.200 | | |
| Total | 210715797.756 | 40 | | | |

leaves was achieved using various analytical methods, including phytochemical screening, TLC, determination of flavonoid and phenolic levels, and FTIR and LC-MS analyses. Further confirmation can be obtained using high-performance liquid chromatography (HPLC) and advanced methods such as 2D or 3D nuclear magnetic resonance (NMR). The collective results underscore the therapeutic potential of agarwood leaves, attributable to their substantial levels of flavonoids and phenolic compounds,[39] as shown in Table 1. LC-MS analysis of the ethyl acetate fraction showed the presence of several bioactive compounds, including apigenin 7.4'-dimethyl ether, quercetin 3-O-glucuronide (miquelianin), and mangiferin. These compounds are well-known members of the flavonoids and xanthone classes, which are widely reported to possess diverse pharmacological properties.[40] In this study, 10 peaks in the ethyl acetate fraction of agarwood leaves were identified, with a high fit conf % of close to 100%. One of the identified compounds was apigenin 7.4'-dimethyl ether (Peak 8), with a molecular formula of $C_{17}H_{14}O_5$ and a molecular weight of 298.290 g/mol. Another identified compound was suspected to be quercetin 3-O-glucuronide (Miquelianin) (Peak 6), with a molecular formula of $C_{21}H_{18}O_{13}$ and a molecular weight of 478.360 g/mol. Furthermore, mangiferin (Peak 2) had a molecular weight of 423.09 g/mol and a molecular formula of $C_{19}H_{18}O_{11}$. These compounds have been previously observed in the *Aquilaria sinensis* species and are related to cytotoxic activity.[41]

Apigenin derivatives have shown antioxidant, anti-inflammatory, and anticancer activities. Previous studies reported that apigenin effectively inhibited oxidative stress and modulated inflammatory pathways by downregulating NF-κB activation. Quercetin and its glycosides, such as quercetin 3-O-glucuronide, are also recognised as potent xanthine oxidase inhibitors, with strong free radical-scavenging capacity, which contributes to the antihyperuricemic effect. Mangiferin, a naturally occurring xanthone, has been extensively studied for its antioxidant and cytoprotective properties, with potential to inhibit xanthine oxidase activity and reduce serum uric acid levels in experimental models. The presence of these compounds supports the inhibitory potential of Agarwood leaves against xanthine oxidase, showing broader pharmacological relevance. Furthermore, the identification of similar compounds in *Aquilaria sinensis* reinforces the consistency of phytochemical profiles across all species. These results show the potential of Agarwood leaves as a promising source of bioactive compounds for the development of natural therapies targeting hyperuricemia and oxidative stress-related disorders.[42] LC-MS analysis of the ethyl acetate fraction of agarwood leaves identified flavonoids, specifically quercetin and apigenin, as well as xanthone compounds, including mangiferin. A study also reported similar results using LC-MS to identify compounds in *Aquilaria sinensis* agarwood leaves, including flavonoids such as quercetin.[40] The results showed that both types of agarwood leaves had flavonoids. LC-MS offers several advantages, including high sensitivity, selectivity, resolution, and rapid performance. During the analysis, separation is represented by a chromatogram in which polar compounds typically elute first, followed by less polar compounds. Non-polar compounds show stronger interactions with the non-polar stationary phase, causing longer retention times, while polar compounds elute more quickly in the presence of relatively polar mobile phases. In this study, a methanol–water (8:2) mixture was used as the mobile phase. Methanol, being polar, is effective in dissolving phenolic compounds and flavonoids,

while the non-polar C18 stationary phase allows non-polar compounds to show longer retention times. Consequently, the chromatogram showed multiple peaks at different retention times, indicating the polarity of each compound and interaction with the stationary phase.[43] Insecticidal activity was assessed by GC-MS analysis of *Pyrenacantha staudtii* leaves, which revealed the presence of essential oil components, including *α*-phellandrene, citronellol, hexadecanoic acid, and tetradecanoic acid. The findings demonstrated that the essential oil was effective against *Rhyzopertha dominica* and *Tribolium castaneum*, the two insects that were examined. This resulted from the essential oil's insecticidal activity and the synergistic action of its monoterpene and sesquiterpene hydrocarbon components.[44]

FTIR spectroscopy measures the absorption of infrared radiation by agarwood leaves. In this study, ethanol extract and ethyl acetate fraction were analysed in semi-solid form, and spectra were recorded using an FTIR spectroscopy. Based on the results, ethanol extract showed the presence of hydroxyl (O–H) groups at wave numbers 3225–3450 $cm^{-1}$, (C-H) stretch, Alkyl groups at wave number 2845–2975 $cm^{-1}$, (C-H; Bend, $CH_2/CH_3$) groups at wave number 1415–1475 $cm^{-1}$, and (C-O) groups at wave number 1000–1125 $cm^{-1}$ (Figure 3), while the ethyl acetate fraction had a broad absorption band at 3200–3600 $cm^{-1}$. This showed hydroxyl groups, characteristic of flavonoids or phenolic compounds (Figure 4). Further analysis of the ethyl acetate fraction showed additional functional groups, including aromatic C–H stretching vibrations at 3000–2855 $cm^{-1}$ and aliphatic C–H bending vibrations at 1470–1415 $cm^{-1}$. The presence of sulfonyl ($SO_2$) groups was also confirmed by absorption bands at 1065–1030 $cm^{-1}$. A previous study on agarwood leaves extract from Malaysia similarly reported an O–H stretching band at 3323.61 $cm^{-1}$, confirming the presence of phenolic compounds and supporting LC-MS analysis. These results suggest that the compounds responsible for the antihyperuricaemic activity of agarwood leaves are flavonoids and phenolic derivatives.[45]

## Conclusion

In conclusion, this study shows that both the ethanol extract and ethyl acetate fraction of agarwood leaves contain flavonoids that inhibit xanthine oxidase. The plant extract exhibited significant xanthine oxidase activity and could be investigated as a lead in drug development as a xanthine oxidase inhibitor for the management of hyperuricemia.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

## Acknowledgments

The author would like to thank the Doctoral Programme in Pharmacy at Padjajaran University for providing lectures and study facilities. Additionally, the author would like to thank Bhakti Kencana University, the Adhi Guna Kencana Foundation, and the Research and Community Service Directorate of Bhakti Kencana University for their financial support for research and publication, as outlined in the Rector's Decision Letter No. 068/01/UBK/VI/2025 regarding the Recipients of Internal Research Grants.

## References

1. Wang Y, Hussain M, Jiang Z, Wang Z, Gao J, Ye F, Mao R, Li H. Aquilaria species (Thymelaeaceae) distribution, volatile and non-volatile phytochemicals, pharmacological uses, agarwood grading system, and induction methods. Molecules 2021; 26. doi: 10.3390/molecules26247708
2. Chandrasekara A, Josheph Kumar T. Roots and tuber crops as functional foods: A review on phytochemical constituents and their potential health benefits. Int J Food Sci 2016; 2016. doi: 10.1155/2016/3631647
3. Wang HN, Dong WH, Huang SZ, Li W, Kong FD, Wang H, Wang J, Mei WL, Dai HF. Three new sesquiterpenoids from agarwood of *Aquilaria crassna*. Fitoterapia 2016; 114: 7–11.
4. Lee HY, Lee JS, Kim HG, Kim WY, Lee SB, Choi YH, Son CG. The ethanol extract of *Aquilariae lignum* ameliorates hippocampal oxidative stress in a repeated restraint stress mouse model. BMC Complement Altern Med 2017; 17: 397.
5. Wang S, Yu Z, Wang C, Wu C, Guo P, Wei J. Chemical constituents and pharmacological activity of agarwood and aquilaria plants. Molecules 2018; 23. doi: 10.3390/molecules23020342
6. Chen L, Liu Y, Li Y, Yin W, Cheng Y. Anti-Cancer Effect of Sesquiterpene and Triterpenoids from Agarwood of *Aquilaria sinensis*. Molecules 2022; 27. doi: 10.3390/molecules27165350
7. Halliwell B, Gutteridge JMC. Free Radicals in Biology and Medicine. Oxford University Press, 2015. . doi: 10.1093/acprof:oso/9780198717478.001.0001
8. Suhardiman A-, Ketut AI, Ika KS, Titin R. Activity of Gaharu (*Aquilaria malaccensis* Lam) Leaves Extract and Fraction as Herbal Drug Against α-Glucosidase Inhibition. Int J Res Pharm Sci 2022; 13: 35–41.
9. Barkas F, Elisaf M, Liberopoulos E, Kalaitzidis R, Liamis G. Uric acid and incident chronic kidney disease in dyslipidemic individuals. Curr Med Res Opin 2018; 34: 1193–9.
10. Sungthong B, Manok S, Sato H, Sato VH. Effects of *Aquilaria crassna* on xanthine oxidase activity in vitro and hyperuricemic mice. Indian J Pharm Sci 2016; 78: 547–52.
11. Niessen WMA. Liquid Chromatography– Mass Spectrometry. Third Edit. London: Taylor and Francis Group, LLC, 2006. .
12. Smith BC. Fundamentals of Transform Fourier Spectroscopy Infrared. 2nd ed. London: CRC Press, 2011. .
13. Aris S, Hana H, Asep R, Aiyi A. Cytotoxic activities of extract and fraction of agarwood leaves (*Aquilaria malaccensis* Lam) as herbal medicine on MCF-7 cancer cells. Int J Res Pharm Sci 2020; 11: 1012–8.
14. J.Houghton P, Raman A. Crude fractionation. Labaratory Handb Fractionation Nat Extr 1998; 54–5.
15. Eissa MA, Hashim YZHY, Abdul Azziz SSS, Salleh HM, Isa MLM, Abd Warif NM, Abdullah F, Ramadan E, El-Kersh DM. Phytochemical Constituents of *Aquilaria malaccensis* Leaf Extract and Their Anti-Inflammatory Activity against LPS/IFN-γ-Stimulated RAW 264.7 Cell Line. ACS Omega 2022. doi: 10.1021/acsomega.2c00439
16. Juanda D, Fidrianny I, Wirasutisna KR, Insanu M. Evaluation of xanthine oxidase inhibitory and antioxidant activities of three organs of idat (*Cratoxylum glaucum* korth.) and correlation with phytochemical content. Pharmacogn J 2021; 13: 971–6.
17. Eissa MA, Hashim YZHY, Mohd Nasir MH, Nor YA, Salleh HM, Isa MLM, Abd-Azziz SSS, Abd Warif NM, Ramadan E, Badawi NM. Fabrication and characterization of Agarwood extract-loaded nanocapsules and evaluation of their toxicity and anti-inflammatory activity on RAW 264.7 cells and in zebrafish embryos. Drug Deliv 2021; 28: 2618–33.
18. Aris S, Asep R, Dewi K, Aiyi A. Cytotoxic Test of Fraction of Gaharu Leaves (*Aquilaria Malaccensis*) on Cervic Cancer Cells (Hela Cells) Using MTT Assay Method. Int J Res Pharm Sci 2021; 12: 1624–31.
19. Dahham SS, Tabana YM, Iqbal MA, Ahamed MBK, Ezzat MO, Majid ASA, Majid AMSA. The anticancer, antioxidant and antimicrobial properties of the sesquiterpene β-caryophyllene from the essential oil of *Aquilaria crassna*. Molecules 2015; 20: 11808–29.
20. Batubara R, Wirjosentono B, Siregar AH, Harahap U, Tamrin. Chemical Compounds and Antioxidant Potential in Hot Water Extract of Cultivated Agarwood (*Aquilaria malaccensis*) Lamk Leaves. IOP Conf Ser Earth Environ Sci 2021; 830. doi: 10.1088/1755-1315/830/1/012070
21. Houghton PJ, Raman A. Chromatographic fractionation procedures. Lab Handb Fractionation Nat Extr 1998; 66–81.
22. Kowalska T. Botanicals – Its Versatile Potential and Selected

Applications. Molecules 2022; 27: 6607.
JD, Morton DW. Anti-Inflammatory and Antimicrobial Activities of Extracts from Medicinal Plants via High-Performance. Molecules 2023; 28: 7346.
24. Riley R, Chapman V. Antioxidant. In: . Nature. Newcastle: Department of Metallurgy, King's College, University of Durham, Newcastle, 1958. ; 1199–200.
25. Abou Baker DH. An ethnopharmacological review on the therapeutical properties of flavonoids and their mechanisms of actions: A comprehensive review based on up to date knowledge. Toxicol Reports 2022; 9: 445–69.
26. Matić P, Sabljić M, Jakobek L. Validation of Spectrophotometric Methods for the Determination of Total Polyphenol and Total Flavonoid Content. J AOAC Int 2017; 100: 1795–803.
27. Batubara R, Wirjosentono B, Siregar AH, Harahap U, Tamrin. Bioactive compounds of ethanol extract from agarwood leaves (*Aquilaria malaccensis*) and antimicrobial activity against bacteria and fungi growing on the skin. Biodiversitas 2021; 22: 2884–90.
28. Xue H, Xu M, Gong D, Zhang G. Mechanism of flavonoids inhibiting xanthine oxidase and alleviating hyperuricemia from structure–activity relationship and animal experiments: A review. Food Front 2023; 4: 1643–65.
29. Okolie NP, Falodun A, Davids O. Evaluation of the Antioxidant Activity of Root Extract of Pepper Fruit (*Dennetia tripetala*), and It'S Potential for the Inhibition of Lipid Peroxidation. African J Tradit Complement Altern Med 2014; 11: 221–7.
30. Rasouli H, Hosseini-Ghazvini SMB, Adibi H, Khodarahmi R. Differential α-amylase/α-glucosidase inhibitory activities of plant-derived phenolic compounds: A virtual screening perspective for the treatment of obesity and diabetes. Food Funct 2017; 8: 1942–54.
31. Bolognesi A, Bortolotti M, Battelli MG, Polito L. Hyperuricaemia, xanthine oxidoreductase and ribosome-inactivating proteins from plants: The contributions of fiorenzo stirpe to frontline research. Molecules 2017; 22. doi: 10.3390/molecules22020206
32. Hofman P, Venissac N, Hofman V, Mouroux J. Molecular modeling of flavonoids that inhibits xanthine oxidase. Biochem Biophys Res Commun 2002; 27: 167–72.
33. Numan Aziz, Jamil RT. Biochemistry, Xanthine Oxidase. Treasure Island: StatPearls Publishing, 2023. .
34. Kapoor N, Saxena S. Potential xanthine oxidase inhibitory
23. Jović MD, Agatonovic-Kustrin S, Ristivojević PM, Trifković activity of endophytic *Lasiodiplodia pseudotheobromae*. Appl Biochem Biotechnol 2014; 173: 1360–74.
35. Yan H, Li L, Jiang X, Li S, Chang Z, Fei X, Li Z. Studies on the Anti-Gouty Arthritis and Anti-hyperuricemia Properties of Astilbin in Animal Models. Open Chem 2020; 18: 207–14.
36. Fam AG. Difficult gout and new approaches for control of hyperuricemia in the allopurinol-allergic patient. Curr Rheumatol Rep 2001; 3: 29–35.
37. Shen M, Zhang J, Qian K, Li C, Xu W, Gu B, Wang X, Ren Q, Yang L, Yuan H, Su D, Chen X. Febuxostat in the treatment of gout patients with low serum uric acid level: 1-year finding of efficacy and safety study. Clin Rheumatol 2018; 37: 3107–13.
38. Emerson RW. ANOVA Assumptions. J Vis Impair Blind 2022; 116: 585–6.
39. Eissa MA, Hashim YZHY, El-Kersh DM, Abd-Azziz SSS, Salleh HM, Isa MLM, Warif NMA. Metabolite profiling of *Aquilaria malaccensis* leaf extract using liquid chromatography-Q-TOF-mass spectrometry and investigation of its potential antilipoxygenase activity in-Vitro. Processes 2020; 8. doi: 10.3390/pr8020202
40. Li Q, Wei P, Li Y, Fu Y. Optimization of Extraction Process and Analysis of Biological Activity of Flavonoids from Leaves of Cultivated 'Qi-Nan' Agarwood. Molecules 2024; 29. doi: 10.3390/molecules29081828
41. Wisutthathum S, Kamkaew N, Inchan A, Chatturong U, Paracha TU, Ingkaninan K, Wongwad E, Chootip K. Extract of *Aquilaria crassna* leaves and mangiferin are vasodilators while showing no cytotoxicity. J Tradit Complement Med 2019; 9: 237–42.
42. Chen L, Teng H, Xie Z, Cao H, Cheang WS, Skalicka-Woniak K, Georgiev MI, Xiao J. Modifications of dietary flavonoids towards improved bioactivity: An update on structure–activity relationship. Crit Rev Food Sci Nutr 2018; 58: 513–27.
43. Chawla G, Ranjan C. Principle, Instrumentation, and Applications of UPLC: A Novel Technique of Liquid Chromatography. Open Chem J 2016; 3: 1–16.
44. Falodun A, Siraj R, Choudhary MI. GC-MS analysis of insecticidal leaf essential oil of *Pyrenacantha staudtii* Hutch and Dalz (Icacinaceae). Trop J Pharm Res 2009; 8: 139–43.
45. Jamahseri NF, Rodhi MNM, Zulkarnaini N., Husain NC, Masruddin AFS. FTIR Analysis Of Phenolic Compound As Pancreatic Lipase Inhibitor From Inoculated *Aquilaria malaccensis*. Malaysian J Anal Sci 2014; 18: 683–9.