Evaluation of phytochemicals, *in vitro* antioxidant and antidiabetic activities of *Aquilaria malaccensis* Lam.

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Abstract

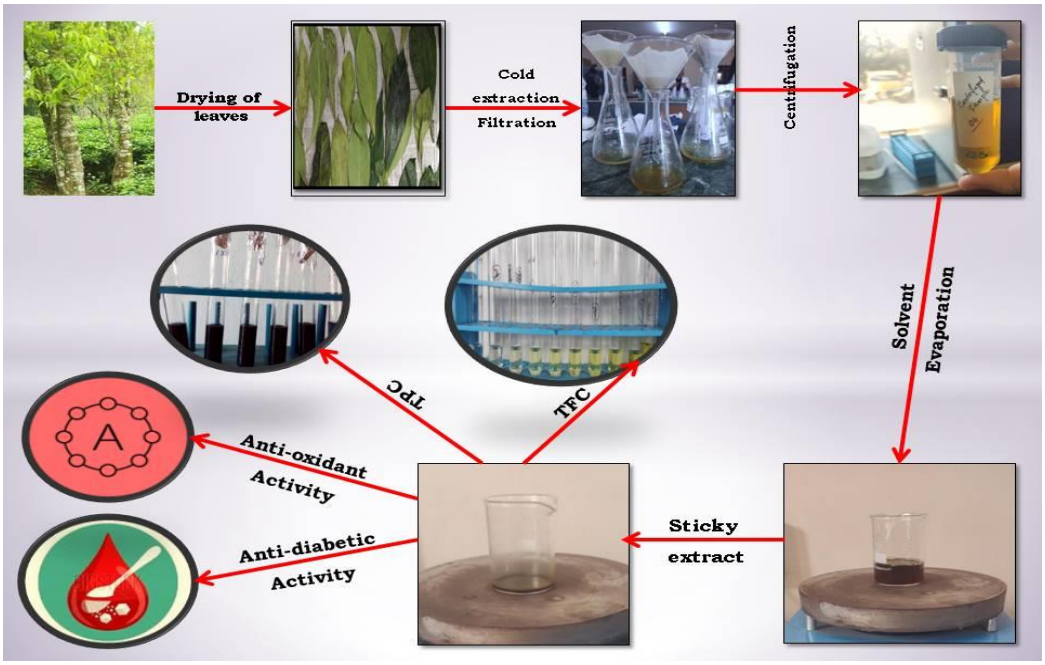
The study focuses on the evaluation of polyphenols, in vitro antioxidant and antidiabetic activities of methanolic extract from the leaves of Aquilaria malaccensis Lam, collected from Assam, India. The leaves underwent 70% methanolic extraction, followed by analysis of total phenolic content (TPC) using the Folin-Ciocalteu method and total flavonoid content (TFC) via the aluminum chloride colorimetric method. The study aimed to investigate the potential bioactive properties of polyphenols present in the plant extract. The antioxidant capacity was assessed through the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay and the antidiabetic potential was evaluated using the α-amylase inhibitory assay. Phytochemical analysis confirmed the presence of polyphenols, flavonoids, saponins, tannins and terpenes, highlighting the rich phytochemical profile of the extract.

The results showed that the methanolic extract had a TPC of 66.26 ± 0.667 µg GAE/mL and a TFC of 134.85 ± 0.776 µg/mL. The extract demonstrated antioxidant activity with a maximum inhibition of 74.23% at 200 µg/mL. In terms of antidiabetic activity, the extract achieved 78.24% inhibition of α-amylase activity at 600 µg/mL. These findings suggest that the Aquilaria malaccensis Lam. leaves possess antioxidant and antidiabetic properties, positioning the plant as a potential source for natural therapeutic agents against oxidative stress and diabetes.

Keywords: *Aquilaria malaccensis* Lam leaves, methanolic extract, polyphenols, antioxidant, antidiabetic activity.

Introduction

Aquilaria malaccensis Lam. is a tropical tree species from the Thymelaeaceae family known by the common name "agarwood". It can be found mostly in Southeast Asian nations including Malaysia, Indonesia, Thailand and Vietnam. Three Aquilaria species: *Aquilaria khasiana*, *Aquilaria macrophylla* and *Aquilaria malaccensis*, can be found in India. The Nicobar Islands is the only home of *A. macrophylla*, whereas the Khasi Hills in Meghalaya are the sole habitat for *A. khasiana*. This species is also referred to locally by several names including Aloe wood, Eagle wood, Sashi, Gaharu and Agaru. The foothills of the northeastern region, which includes Assam and West Bengal, are where *A. malaccensis* is primarily found. Basically, it is a fast-growing tree that can be found growing anywhere from the Papua New Guinea rain forests to the slopes of the Himalayas[1].



Graphical Abstract

It is a massive evergreen tree with white blossoms that is about 15–40 m tall and 0.6–2.5 m in diameter. It reproduces through seeds and is present in the forest growing alongside different plants, shrubs and tree species[2]. Agarwood is in great demand throughout Asia and the Middle East for medicine, incense and perfumes. The aromatic resinous heartwood of agarwood, which is highly prized, is a key component in all of them[3]. *A. malaccensis* is one of nine Aquilaria species in the Thymelaeaceae family that produce agarwood and it is also the most common[4].

Aquilaria malaccensis Lam., known for its resinous heartwood, has been widely studied for its rich polyphenolic content, which contributes to its antioxidant and antidiabetic properties. Polyphenols, which play a critical role in neutralizing free radicals, have been identified as major contributors to the plant's bioactivity[5]. These compounds help to reduce oxidative stress, which is often associated with various chronic conditions, including diabetes. Sarmah et al[12] emphasized the role of bioactive compounds like agarospirol in modulating metabolic pathways, suggesting that *A. malaccensis* could have potential antidiabetic effects through the regulation of glucose levels.

Additionally, Eissa et al[5] reported that ethanolic extracts of *A. malaccensis* leaves demonstrated significant antioxidant activity *in vitro*, primarily due to the presence of flavonoids and phenolic acids. This activity may complement the plant's antidiabetic properties by mitigating oxidative stress, which plays a key role in the development of diabetes. These findings support further investigation into the polyphenolic composition of *A. malaccensis* and its potential as a natural therapeutic agent for managing oxidative stress and metabolic disorders.

The aim of this research is to evaluate the polyphenolic content and to investigate the *in vitro* antioxidant and antidiabetic activities of methanolic extracts from the leaves of *Aquilaria malaccensis*. This study seeks to establish a correlation between the polyphenolic profile and the observed bioactivities, providing insights into the therapeutic potential of the plant's leaves. Such findings could support the development of natural antioxidant and antidiabetic agents.

Material and Methods

Sample collection and preparation of Methanolic Extract: The fresh, disease free leaves of *Aquilaria malaccensis* Lam were collected from Golaghat district, Assam. Leaves were transported to the Biotechnology laboratory, Assam down town University on the same day and were cleaned and washed under running tap water. The samples were shade-dried for about 6 days and later they were weighed and grinded with a grinder to make fine powder. Cold extraction was carried out by weighing 10gms of the samples soaked with 70% methanol for 72 hours and filtered using Whatmann no. 1 paper followed by centrifugation at 5000 rpm for 5 mins. The filtrate was stored in a clean beaker and the solvent was entirely removed using a magnetic stirrer hot plate, resulting in a sticky mass. The crude extracts were then weighed and stored at 4°C until further analysis. Plant leaves samples were also identified at Program of Botany, Assam down town University by a Taxonomist.

Phytochemical Screening: The presence of phytoconstituents was analyzed using freshly prepared reagents. All the used glass wares were thoroughly cleaned before the experimental works. The extract was analyzed for the presence of carbohydrates, terpenes, steroids, phenols, flavonoids, tannins, saponin etc.[8]

Determination of Total Phenolic Content: The total phenolic content of the extract was quantified using the Folin–Ciocalteu reagent, following the protocol established by Singleton and Rossi[14] using gallic acid as standard. A standard gallic acid solution (100 µg/mL) and a 10% Folin-Ciocalteu reagent solution were prepared, along with a 7% Na_2CO_3 solution. Different concentrations of gallic acid (200-1000 µg/mL) were mixed with methanol up to 1 mL.

The mixture was incubated at room temperature for 5 minutes. Then 5 mL of Folin-Ciocalteu reagent and 4 mL of Na_2CO_3 solution were added respectively, resulting in a final volume of 10 mL. The mixture was allowed to stand for 2 hr with intermittent shaking at room temperature. Absorbance was measured at 760 nm, with a blank as the reference.[10]

Determination of Total Flavonoid Content: The total flavonoid content was assessed using the aluminum chloride ($AlCl_3$) method as described by Zhishen et al[20] with rutin employed as the standard. Various concentrations of the Rutin working standard (0.2mL-1.0 mL) were pipetted and made up to 1 mL with distilled water. Following this, 0.03 mL of 5% $NaNO_2$ was added and incubated for 5 minutes at 25°C. Subsequently, 0.03 mL of 10% $AlCl_3$ was added. After an additional 5 minutes, the reaction mixture was treated with 0.2 mL of 1 mM NaOH. Absorbance was measured at 510 nm.

Determination of Anti-Oxidant Activity: DPPH scavenging assay was used to test the scavenging activity. Different concentrations of plant extracts (50, 100, 150 and 200 µg/mL) were combined with 2 mL of methanolic DPPH solution and incubated for 30 minutes. The DPPH free radical scavenging activity was then measured at 517 nm with ascorbic acid as the standard.[12]

The formula used to calculate free radical scavenging activity is:

$$\% \text{ Inhibition} = [(AB - AA) / AB] \times 100$$

where AB is the absorbance of the blank solution and AA is the absorbance of the sample. This formula quantifies the inhibition percentage of free radicals by the sample.

Determination of Anti-Diabetic Activity: A starch solution (0.1% w/v) was formulated by solubilizing 0.1g of potato starch in 100 mL of 16 mM sodium acetate buffer. An enzyme solution was made by dissolving 27.5 mg of alpha-amylase in 100 mL of distilled water. The colorimetric reagent was produced by combining sodium potassium tartrate with a 96 mM solution of 3,5-dinitrosalicylic acid. Both the control and plant extract were introduced to the starch solution and incubated with the alpha-amylase solution under alkaline conditions at 25°C. The reaction was monitored over a duration of 10 minutes. The production of maltose was quantified through the reduction of 3,5-dinitrosalicylic acid to 3-amino-5-nitrosalicylic acid, with absorbance measured spectrophotometrically at 540 nm.[12,13]

Percentage of inhibition was calculated using the formula:

$$\% \text{ Inhibition} = [(AB_{PC} - AA) / AB_{PC}] \text{ X } 100$$

where AB_{PC} is the absorbance of the blank solution and AA is the absorbance of the sample. All the test was performed in triplicate and results were expressed as mean and standard deviation.

Results and Discussion

Phytochemical Screening: The methanolic extract of *Aquilaria malaccensis* Lam. leaves was found to contain significant phytochemicals, as in table 1. These bioactive compounds contribute to various pharmacological properties.

Table 1
Phytochemical Screening

Phytochemical	Result
Terpene	+
Carbohydrate	+
Tannin	+
Proteins	-
Flavonoid	+
Steroid	-
Phenol	+
Saponin	+

+ Present, - Absent

A similar investigation by Batubara et al[2] on *Aquilaria malaccensis* leaves from Indonesia revealed the presence of alkaloids, flavonoids, tannins and saponins. The absence of steroids in both studies confirms that saponins and tannins are more prevalent in Aquilaria leaves[14]. Further comparison with Surjanto et al[15] confirmed the presence of saponins and tannins in *Aquilaria malaccensis* leaves, consistent with the findings of this research. These comparisons highlight that the plant exhibits a robust array of secondary metabolites contributing to its medicinal value.

The presence of saponins and terpenes has been linked to anti-inflammatory, antioxidant and antimicrobial activities which align with the traditional uses of *Aquilaria malaccensis* for health purposes. Thus, the phytochemical profile confirms the pharmacological potential of this species, though the absence of alkaloids in this study indicates geographic and environmental factors likely to influence the exact profile.

Total Phenolic Content: The total phenolic content (TPC) of the methanolic extract of *Aquilaria malaccensis* was determined to be 66.26 ± 0.667 µg GAE/mg, highlighting the extract's potential as a source of phenolic compounds. This result is comparable to findings from recent studies. Oven-dried *Aquilaria malaccensis* leaves extracted with 70% ethanol exhibited total phenolic content values ranging from 52.98 to 85.15 mg GAE/g. Similarly, Delica-Balagot et al[3] determined the phenolic content in dehydrated *Aquilaria malaccensis* leaf samples to be ranged from 48.84–56.41 mg GAE/g. The observed variations in phenolic content across studies can be attributed to differences in extraction methods, solvent types and processing conditions.

Total Flavonoid Content: In the present study, the total flavonoid content of the methanolic extract of *Aquilaria malaccensis* was determined to be 134.8467 ± 0.776 µg/mL. Oven-dried *Aquilaria malaccensis* leaves extracted with 70% ethanol exhibited total flavonoid content values ranging from 2180.97 to 3733.45 QUE ppm. Likewise, Delica-Balagot et al[3] reported a flavonoid content of 524.33 ± 0.04 mmol TEAC/g in dehydrated *Aquilaria malaccensis* leaf samples.

DPPH Radical Scavenging Assay: In the present study, the methanolic extract of *Aquilaria malaccensis* demonstrated an antioxidant activity with a DPPH inhibition of 74.23% (Fig. 1A) at a concentration of 200 µg/mL and an IC50 value of 85.9733±0.0173 µg/mL at 95% confidence level (Fig. 1B). These results are compared with findings from recent studies. Aqueous extracts of *Aquilaria malaccensis* leaves showed a DPPH inhibition of 78.45% at a concentration of 200 µg/mL, with an IC50 value of 84 µg/mL. Similarly, Ridwanto et al[11] found that methanolic extracts of *Aquilaria malaccensis* bark exhibited a DPPH inhibition with an IC50 value of 94.59 µg/mL. Some researchers used aqueous extraction, while Ridwanto et al[11] employed methanolic extraction, highlighting the impact of solvent choice on antioxidant activity.

Antidiabetic Assay: The methanolic extracts of *Aquilaria malaccensis* Lam. leaves showed significant inhibitory effect on alpha amylase activity *in vitro* in a dose dependent manner (Fig. 2A), inhibiting α-amylase by 78.24% at a concentration of 600 µg/mL, with an IC50 value of 369.68±0.0113 µg/mL at 95% confidence level (Fig. 2B).

These results are compared with findings from recent studies. Zulkifle[21] found that methanolic extracts of *Aquilaria malaccensis* leaves showed α-amylase inhibitory activity with IC50 values of 425.09 µg/mL.

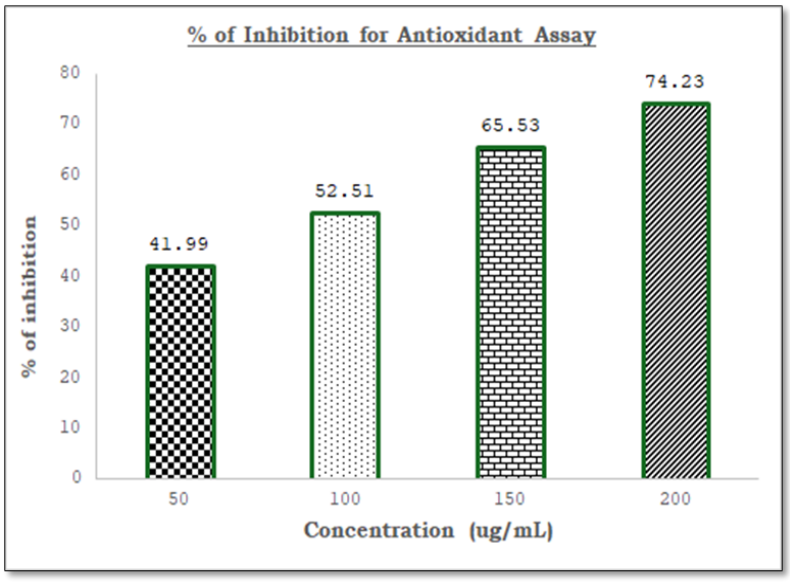


Fig. 1A: Showing antioxidant inhibition

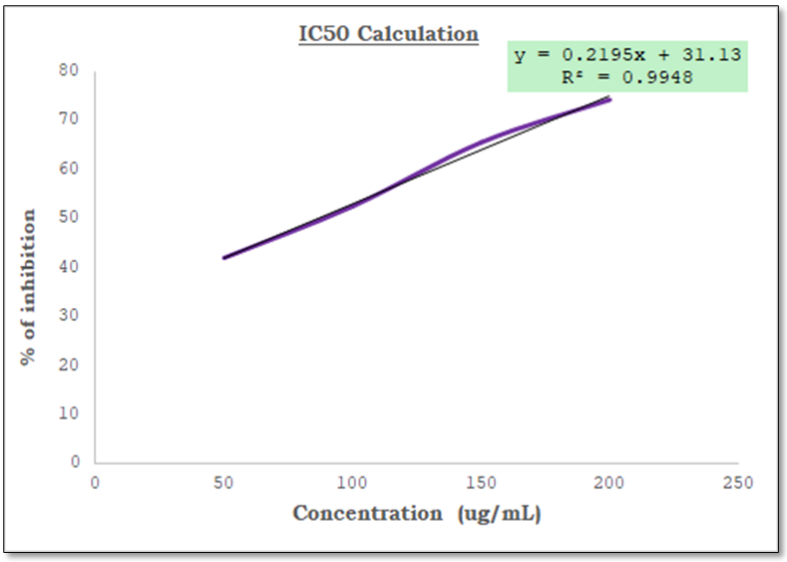


Fig. 1B: Showing IC50 calculation

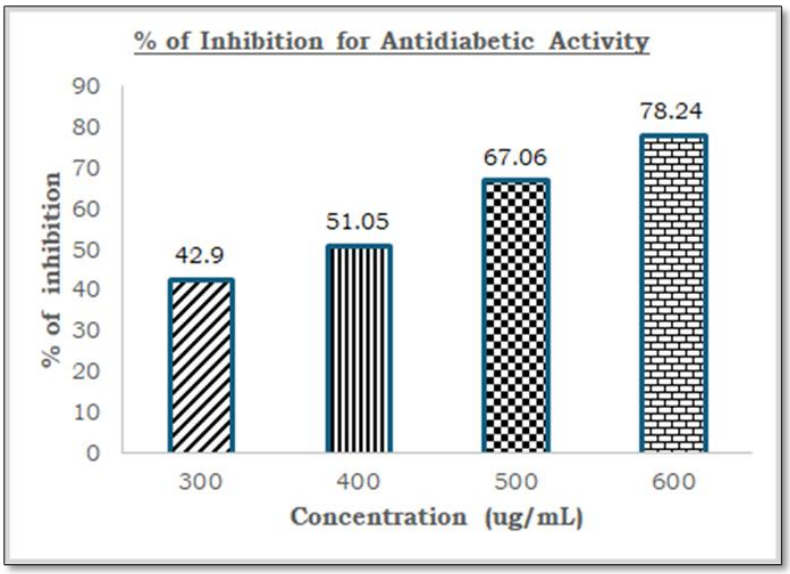


Fig. 2A: Showing anti-diabetic inhibition

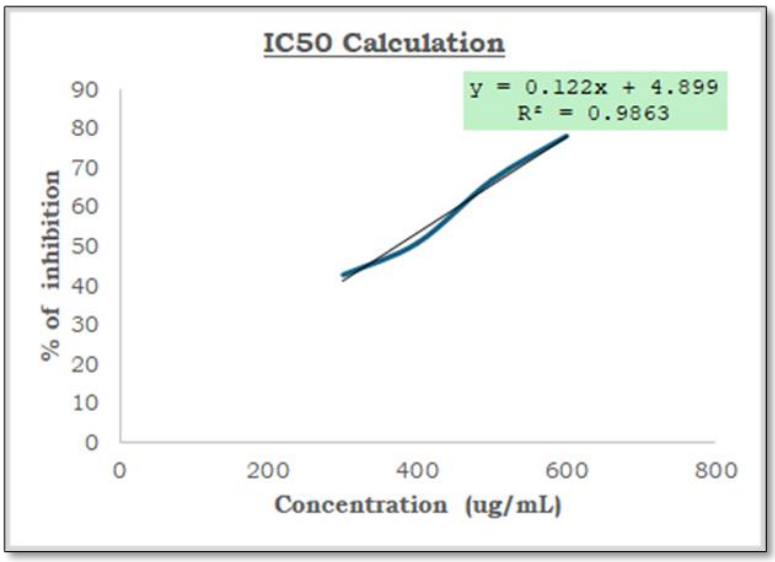


Fig. 2B: Showing IC50 calculation

Nur Liyana et al[10] also conducted the same alpha amylase inhibitory assay on methanolic extracts of *Aquilaria malaccensis* Lam. collected from Pahang, Malaysia and revealed that the samples with 400, 600, 800 and 1000 µg/mL concentration showed % inhibition of 50.32, 66.67, 70.95 and 78.48% against alpha-amylase respectively and IC50 value of 397.25 µg/mL.

Conclusion

The methanolic extract of *Aquilaria malaccensis* Lam. leaves has demonstrated significant potential as a natural source of bioactive compounds with antioxidant and antidiabetic properties. The high total phenolic and flavonoid content, along with strong DPPH radical scavenging activity, indicates the extract's effectiveness in neutralizing free radicals, thus contributing to its antioxidant capability. Furthermore, the extract's ability to inhibit α-amylase activity suggests a promising role in managing hyperglycemia and type 2 diabetes. These findings underscore the therapeutic potential of *A. malaccensis* as a natural antioxidant and antidiabetic agent.

Future studies could focus on isolating and characterizing the specific compounds responsible for these bioactivities, offering a path toward the development of novel treatments for oxidative stress and diabetes management. This research reinforces the value of traditional medicinal plants in modern therapeutic applications, particularly in the development of plant-based drugs.

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