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Extraction, Chemical Characterization, and Evaluation of the Antidiabetic Potential of *Aquilaria malaccensi Root Extracts*: An In Vitro and Molecular Docking Study

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Abstract: The present study aims to investigate the phytochemical composition of ethanolic and methanolic root extracts of Aquilaria malaccensis through extraction, isolation, and characterization of bioactive compounds. Aquilaria malaccensis is a medicinally significant plant renowned for its traditional therapeutic applications. The major isolated constituents were identified as flavonoid-related compounds. The ethanol and methanol root extracts and isolated compounds like MAME-02 and EAME-01 were evaluated for their in vitro antidiabetic activity. At 500 µg/mL, EAME-01 showed the highest inhibitory activity (98.6%), followed by acarbose (96.3%) and MAME-02 (82.34%). The ethanol extract (65.45%) was more active than the methanol extract (52.38%). The structures of the bioactive phytoconstituents were confirmed by spectroscopy analysis. Finally, the molecular docking studies were caried out using AutoDock Vina with the insulin receptor (IR, PDB ID: 1IR3) of isolated compound EAME-01 and EAME-02 and revealed favorable binding energies of −10.89 and −11.87 kcal/mol, respectively. EAME-01 exhibited the strongest binding, supported by hydrogen bonding, hydrophobic interactions, and metal coordination. MAME-02 formed four hydrogen bonds and additional stabilizing interactions with key amino acid residues and magnesium ions, suggesting potential insulin receptor-binding activity.

Keywords: Aquilaria malaccensis, In Vitro antidiabetic activity, Molecular docking studies, Insulin receptor.

INTRODUCTION

Plants have been extensively utilized in traditional medicine, perfumery, and the food industry as flavoring agents and natural preservatives. Various plant-derived extracts have also been traditionally employed in the management of a wide range of acute and chronic diseases [1]. These therapeutic properties are attributed to the presence of diverse bioactive phytoconstituents, including alkaloids, steroids, tannins, glycosides, volatile and fixed oils, resins, phenolic compounds, and flavonoids, which are distributed throughout different plant parts and contribute to their potential pharmacological and biological activities [2]. Natural medicines remain an important component of primary healthcare in developing countries and are increasingly gaining recognition in developed nations owing to their accessibility, affordability, and potential therapeutic benefits. More than 80% of the global population is reported to rely on traditional medicine for primary healthcare, with plant-derived preparations serving as a major component. Medicinal plants represent a valuable source of bioactive compounds used in traditional medicine, nutraceuticals, dietary supplements, pharmaceutical intermediates, and the development of both natural and synthetic drugs [3-6].

Agarwood is a commercially valuable non-timber forest product obtained from the resinous heartwood of Aquilaria species, with extensive applications in traditional medicine and the fragrance industry. The dark, fragrant resin is highly prized for its distinctive aroma and economic significance [7]. Among the principal agarwood-producing species, Aquilaria crassna, A. malaccensis, and A. sinensis are widely cultivated in southern China, India, and other Southeast Asian regions and have a long history of use in traditional medicine spanning more than a thousand years [8-10]. Despite its considerable commercial value, agarwood cultivation is constrained by the prolonged period required for the formation of aromatic heartwood, which may take approximately ten years and limit the economic returns for farmers and commercial producers. Consequently, exploring alternative, readily available plant resources, particularly Aquilaria leaves, offers a promising approach to improving resource utilization. These leaves may serve as potential sources of aromatic and bioactive phytoconstituents, thereby expanding the economic and pharmaceutical applications of Aquilaria species [11]. *Aquilaria* spp. are the most valuable and highly fragrant forest products locally known as agarwood, aloes wood, eaglewood, guacharo, kalamabak, or oudh depending on the region [12]. Several genera that might be a source of agarwood production from the family Thymelaeaceae might be endangered due to the deterioration of their natural resources include *Aquilaria, Gonystulus*, and *Gyrinops*, having been listed in Appendix II of the Convention on Internal Trade in Endangered Species of Wild Fauna and Flora [13].



Fig 1. Plant of *Aquilaria malaccensis*

In Malaysia and Indonesia, the resin produced by *Aquilaria* species generally called as a gaharu and *A. malaccensis* known as a main genus to produce agarwood or gaharu. Agarwood has many uses such

as perfumes, incense and medicines[14]. The main markets for these products are in South and East Asia and the Middle East. The identification of the botanical and ecological aspects of this species has been widely identified from a chemical constituent point of view that can be explain its uses as an important and excellent source of pharmaceutical products. Many parts of this plant, including the leaves, skin, seeds, wood and roots are valuable in medicinal properties. It is highly sought after for its resin and essential oils while less has been focused on the health beneficial effects of other parts of the plant despite the various ethnopharmacological evidences. These include antioxidant activities, analgesic, antipyretic, anti-inflammatory, anti-hyperglycaemic, and antimicrobial for various medicinal purposes [15-17].

MATERIALS AND METHODS

All chemicals, reagents, and materials used in this study, including ethanol, methanol, distilled water, extraction and chromatographic solvents, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Whatman filter paper, silica gel, and ascorbic acid, were analytical grade and used as received without further purification. Nutrient agar and other microbiological media were obtained from established suppliers, including Invitrogen, Sigma-Aldrich, and HiMedia Laboratories Pvt. Ltd., Mumbai, India. The principal equipment employed in the experimental procedures included a mechanical grinder, Soxhlet apparatus, maceration assembly, analytical balance, rotary evaporator, and hot-air oven.

Plant collection, extracts, phytochemical screening and Isolation

Fresh roots of *Aquilaria malaccensis* were collected and authenticated by a qualified botanist. The roots were thoroughly washed, shade-dried, powdered, and stored in airtight containers until further use. The powdered root material (200 g) was defatted with petroleum ether using a Soxhlet apparatus, dried at room temperature, and separately extracted with ethanol and methanol by Soxhlet extraction and maceration. The extracts were filtered, concentrated under reduced pressure, dried, weighed, and stored at 4°C until further analysis. The percentage extraction yield was calculated based on the weight of the dried extract relative to the initial weight of the plant material. Preliminary phytochemical screening was conducted using standard procedures to detect the presence of various secondary metabolites, including alkaloids, flavonoids, phenolics, tannins, saponins, glycosides, terpenoids, steroids, carbohydrates, proteins, and reducing sugars. For the isolation of phytoconstituents, aliquots of the crude extracts were dissolved in chloroform and analyzed by thin-layer chromatography (TLC).

The developed plates were visualized under ultraviolet (UV) light and treated with 10% sulfuric acid reagent to identify the separated components. Subsequently, 2 g of each crude extract was subjected to column chromatography using silica gel (2 × 90 cm) as the stationary phase. Elution was performed using mixtures of n-hexane and ethyl acetate in varying proportions, ranging from 2:8 to 4:6, to facilitate the separation of individual constituents. The isolated compounds were collected and purified before being characterized using Fourier-transform infrared (FT-IR) spectroscopy, proton nuclear magnetic resonance (^{1}H NMR) spectroscopy, carbon-13 nuclear magnetic resonance (^{13}C NMR) spectroscopy, and mass spectrometry. The isolated phytoconstituents were designated MAME-02 and EAME-01, and their structures were elucidated using the combined spectroscopic and analytical data.

In Vitro antidiabetic activity

Alpha-Amylase Inhibition Assay:[18] The starch solution (0.1% w/v) was prepared in 16 mM sodium acetate buffer, and the α-amylase solution was prepared in distilled water. Ethanolic and methanolic extracts of *Aquilaria malaccensis*, isolated compounds, and acarbose (standard) were dissolved in 5% DMSO and serially diluted to obtain concentrations of 5–1000 µg/mL. The test and standard solutions (500 µL) were mixed with α-amylase solution (0.5 mg/mL) and incubated at room temperature for 10 min. Starch solution (1% w/v) was then added, followed by incubation for another 10 min. DNS reagent was added, and the mixture was heated in a boiling water bath for 5 min. After cooling, distilled water was added, and absorbance was measured at 540 nm. α-Amylase inhibition was expressed as a percentage, and IC_{50} values were determined using linear regression analysis.

Percentage inhibition was calculated as:

% inhibition = [(100- Abs. of sample – Abs. of blank) X 100] Abs blank.

Alpha-Glucosidase Inhibition Assay:[19] The assay was performed by incubating 1 mL of substrate solution (2% w/v maltose or sucrose) with 0.2 mM Tris buffer (pH 8.0) and different concentrations of plant extract at 37°C for 5 min. Subsequently, 1 mL of α-glucosidase (1 U/mL) was added, and the mixture was incubated at 35°C for 40 min. The reaction was terminated by adding 2 mL of 6 N HCl, and absorbance was measured at 540 nm. The percentage inhibition was calculated relative to the control, and the IC_{50} value was determined from the inhibition percentages obtained at five different extract concentrations.

$$\mathbf{I\ \% = (Ac\text{-}As)/Ac\ X\ 100}$$

Where,

Ac is the absorbance of the control and

As is the absorbance of the sample

Molecular Docking Studies [20].

Molecular docking analysis was conducted using the insulin receptor (IR; PDB ID: 1IR3) as the target protein and the selected compounds as ligands. The three-dimensional structures of the ligands were obtained from the PubChem database, while the receptor structure was retrieved from the RCSB Protein Data Bank (PDB). Water molecules and co-crystallized ligands were removed using PyMOL prior to docking. Blind molecular docking was subsequently performed using AutoDock Vina to identify potential binding sites and assess protein–ligand interactions. The complexes exhibiting favorable docking scores were selected for further evaluation.

Statistical Analysis

The results were recorded after repeating the experiments six times. The experimental results were expressed as mean ± standard error (SE) of (3n) measurements. The statistical analysis of the data was carried out using Student's t-test, and the results were considered significant when $p<0.05$.

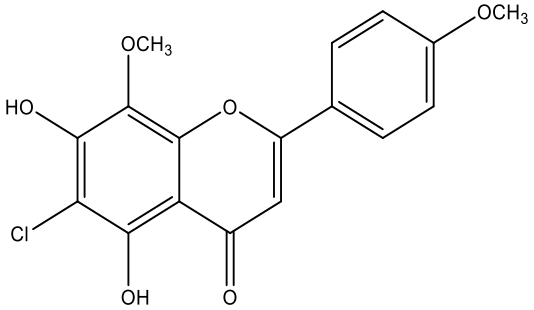
RESULTS AND DISCUSSION

Preliminary Phytochemical Screening of ethanol and methanol Extract of Roots of *Aquilaria malaccensis*. Standard methods were used for the identification of this preliminary phytochemical screening of the both root extracts of *plant* revealed the presence or absence of secondary metabolites. The qualitative analysis showed the presence of Alkaloids, Glycosides, Phytosterols, and Flavonoids compounds in the ethanol and methanol extracts with varying intensities. As shown results, the majority of these secondary metabolites are present in the both extracts are Flavonoids.

Table 1. Preliminary phytochemical Screening.

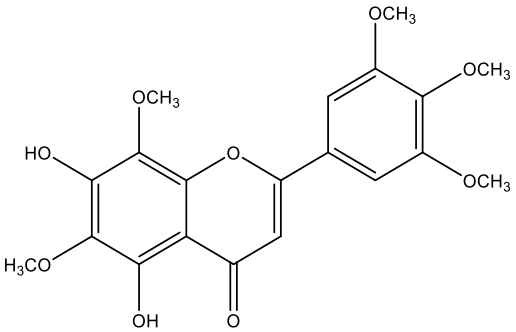
Test	Root of *Aquilaria malaccensis*	
	Methanol Extract	**Ethanol Extract**
Alkaloids	+	+
Glycosides	+	-
Carbohydrates	-	-
Phenolic compounds	-	-
Steroids	-	+
Flavonoids	+++	+++
Terpenes	-	-
Saponins	-	-

Characterization of Isolated Compounds-Roots of *Aquilaria malaccensis*. Identification and analytical, standard spectroscopic methods for investigating the structure of natural products- ethanol (EAME-01) and methanol (MAME-02) root extracts of *Aquilaria malaccensis* comprise nuclear magnetic resonance (NMR), infrared spectroscopy (IR), and these are often combined with mass spectrometry (MS). In physical and chemical characterization, the EAME-01 isolated as pale-yellow colour powder and MAME-02 was isolated as brown colour powdered. The melting point of both the compounds are 181-183^{o}C (EAME-01) and 207-209^{o}C (MAME-02) respectively.



6-chloro-5,7-dihydroxy-8-methoxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one
Chemical Formula: $C_{17}H_{13}ClO_6$
Exact Mass: 348.04

Fig 2. EAME-01



Chemical Formula: $C_{20}H_{20}O_9$
Exact Mass: 404.11

5,7-dihydroxy-6,8-dimethoxy-2-(3,4,5-trimethoxyphenyl)-4*H*-chromen-4-one

Fig 3. MAME-02

Characterization of EAME-01: IR values(cm^{-1}):IR band was observed at 3453(-OH Str, Ar-OH); 3087(-CH Str, Ar-CH); 2976,2899(-CH Str, Alkane); 1727(-C=O Str, Keton group); 1610-C-C Str in Ar-C); 1423(-C=C Str in Ar-C=C), 1078(-C-O Bend). ^{1}H NMR spectrum. It was observed at 12.865(1H, s, Ar-OH); 10.766(1H, s, Ar-OH); 8.253(1H, s, Ar-H); 8.102-8.001(2H, d, Ar-H); 7.869-7.823(2H, d, Ar-H); 3.775-3.683(s, 6H, Ar-OCH_3). Mass (m/z). 348.04g/mole (M^+), 349.14(M+1).

Characterization of MAME-02: IR values(cm^{-1}):IR band was observed at 3532, 3484(-OH Str, Ar-OH); 3102(-CH Str, Ar-CH); 2956,2867(-CH Str, Alkane); 1734(-C=O Str, Keton group); 1602(C-C Str in Ar-C); 1419(-C=C Str in Ar-C=C), 1103(-C-O Bend). ^{1}H NMR spectrum. It was observed at 12.643(1H, s, Ar-OH); 10.276(1H, s, Ar-OH); 8.453(1H, s, Ar-H); 8.195-8.102(2H, d, Ar-H); 3.834-3.732(s, 9H, Ar-OCH_3), 3.587-3.521(s, 6H, Ar-OCH_3). Mass (m/z). 404.11gmol (M^+), 405.21(M+1). The isolated **EAME-01** and **MAME-02** were characterized by IR, ^{1}HNMR and Mass spectral data**.** The results revealed that the isolated compound was identified as a **flavonoid.**

***In-vitro* antidiabetic Activity: Alpha-amylase inhibition activity:**

The α-glucosidase inhibitory activity of MAME-02, EAME-01, and ethanol and methanol root extracts of *Aquilaria malaccensis* was evaluated at 10–500 µg/mL using acarbose as the standard. All samples exhibited concentration-dependent inhibition. At 500 µg/mL, EAME-01 showed the highest inhibitory activity (98.6%), followed by acarbose (96.3%) and MAME-02 (82.34%). The ethanol extract (65.45%) was more active than the methanol extract (52.38%). These findings indicate that EAME-01 possesses promising α-glucosidase inhibitory potential and warrants further investigation as a potential source of antidiabetic compounds.

Table 2. Alpha-amylase inhibition activity of methanol, ethanol extract, EAME-01 and MAME-02.

Conen. (µg/mL)	MAME-02	EAME-01	Ethanol extract	Methanol extract	Acarbose
10	20.25±0.002	24.36±0.022	16.83±2.732	12.64±3.012	23.2±0.023
25	30.64±0.902	31.83±0.212	23.47±1.263	17.23±0.997	38.1±0.053
50	40.34±0.218	43.56±1.002	32.84±3.094	21.42±2.102	54.2±2.042
100	52.34±0.293	55.34±1.543	43.57±1.102	32.56±1.029	60.2±0.012
200	67.43±0.022	71.26±2.001	50.23±0.102	42.12±2.032	70.1±0.103
500	82.34±0.002	98.6±0.322	65.45±0.034	52.38±1.321	96.3±0.001

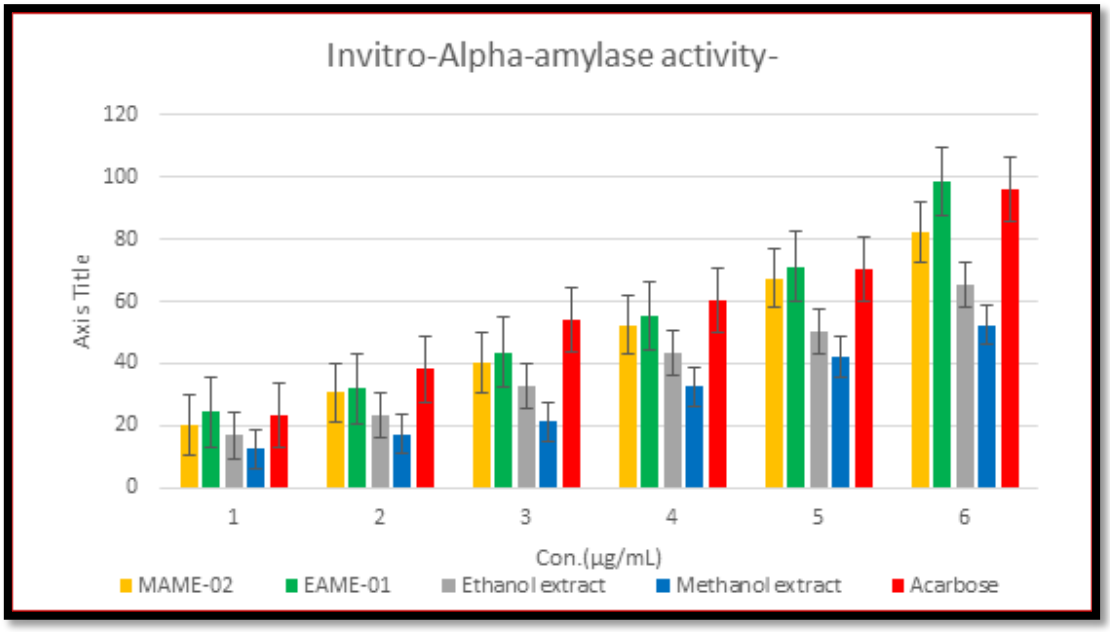


Fig 4. Graphical representation of Alpha-amylase inhibition activity of methanol, ethanol extract, EAME-01, and MAME-02.

Alpha(*α*)-*glucosidase inhibitory activity*: The α-glucosidase inhibitory activity of MAME-02, EAME-01, and ethanol and methanol root extracts of *Aquilaria malaccensis* was evaluated at 10–500 µg/mL using acarbose as the standard. All samples exhibited concentration-dependent inhibition. EAME-01 showed the highest activity among the tested compounds and extracts, followed by MAME-02, the ethanol extract, and the methanol extract. However, acarbose demonstrated the highest overall activity. These findings suggest the potential of EAME-01 for further investigation as a candidate for antidiabetic applications.

Table 3. Alpha-glucosidase % inhibition activity of methanol, ethanol extract, EAME-01, and MAME-02.

Conen. (µg/mL)	MAME-02	EAME-01	Ethanol extract	Methanol extract	Acarbose
10	23.31±1.03	35.19±2.94	27.46±2.98	18.98±0.02	38.43±0.01
25	36.16±.2.02	42.34±1.976	32.75±.1.09	23.54±0.24	56±1.21
50	48.34±1.98	52.43±1.05	41.87±4.12	28.45±1.65	62±0.83
100	60.32±2.05	67.24±2.87	59.76±3.54	42.87±1,04	72±0.05
200	72.34±0.64	82.56±1.93	61.34±032	56.34±0.43	87±0.02
500	89±1.83	102.45±2.65	78±3.24	67.34±0.01	119±0.41

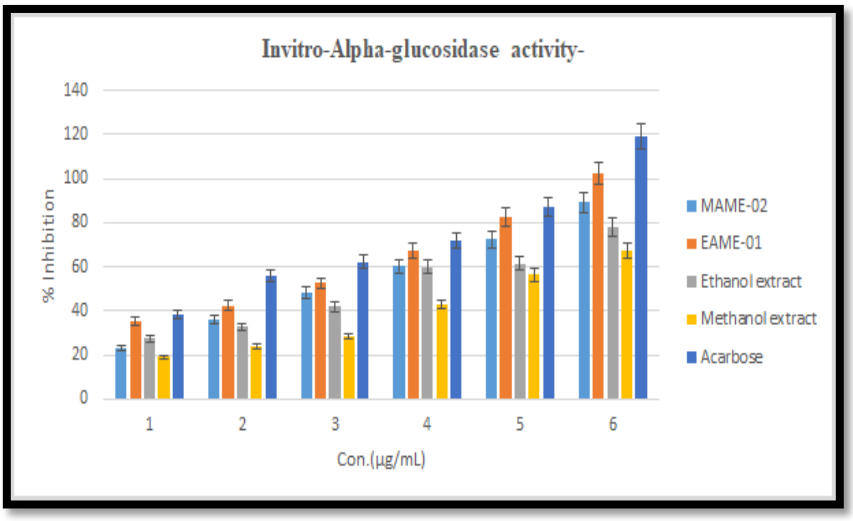


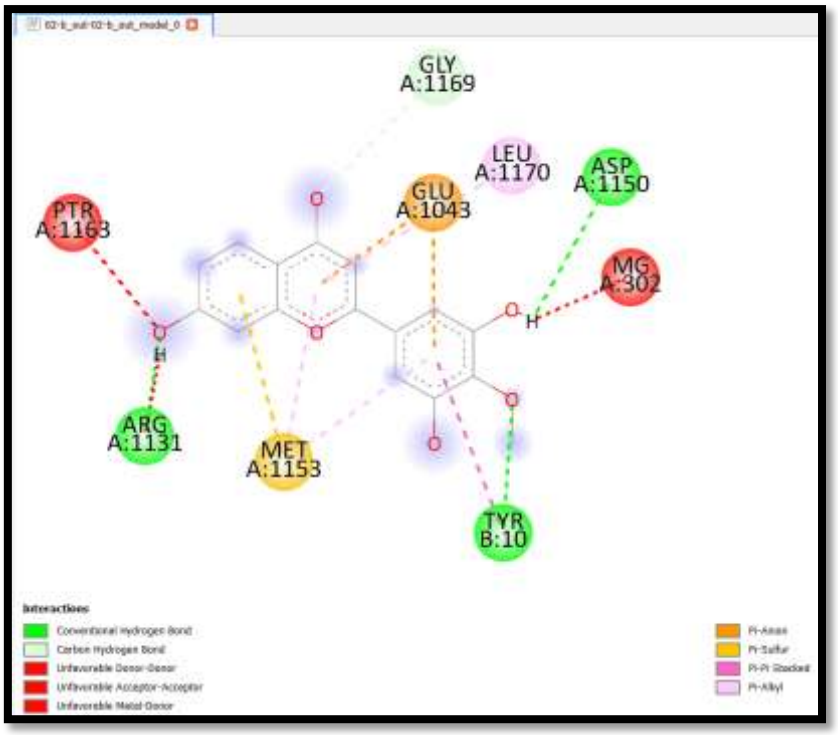
Fig 5. Graphical representation of alpha-glucosidase activity (% Inhibition) activity of methanol, ethanol extract, EAME-01 and MAME-02.

Molecular Docking Studies.

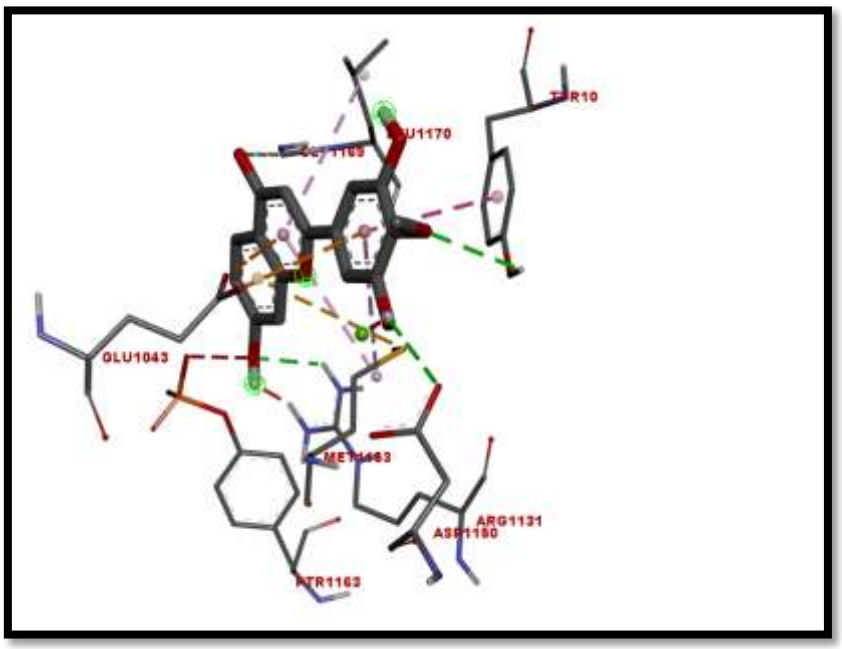
The docking analysis of the designed compounds with the insulin receptor (IR, PDB ID: 1IR3) demonstrated a range of binding affinities between **–10.9 and –11.84 kcal/mol**, with distinct hydrogen bonding and hydrophobic interactions contributing to ligand stabilization. The isolated compounds **MAME-02** and **EAME-01-(Flavonoid related compounds)** showed the most favorable binding, with **EAME-01** (-11.87 kcal/mol) forming three hydrogen bonds with ARG:1131, and ASP:1150 supported by strong hydrophobic and metal interactions involving PTR:1163, MET:1153, GLU:1043, GLY:1169, TYR:10, and MG: 302. Similarly, **MAME-02** (-10.89kcal/mol) established four hydrogen bonds each with residues such as TYR:10, ASP:1150, ASP:1132, PTR:1163, and ARG:1131 stabilized further by interactions with magnesium ions (MG301, MG302) and key hydrophobic residues including GLU:1043, EU:1170, GLY:1163, and MET:1153.

Table 4. Binding energies and interaction details of Isolated compounds EAME-01 and MAME-02.

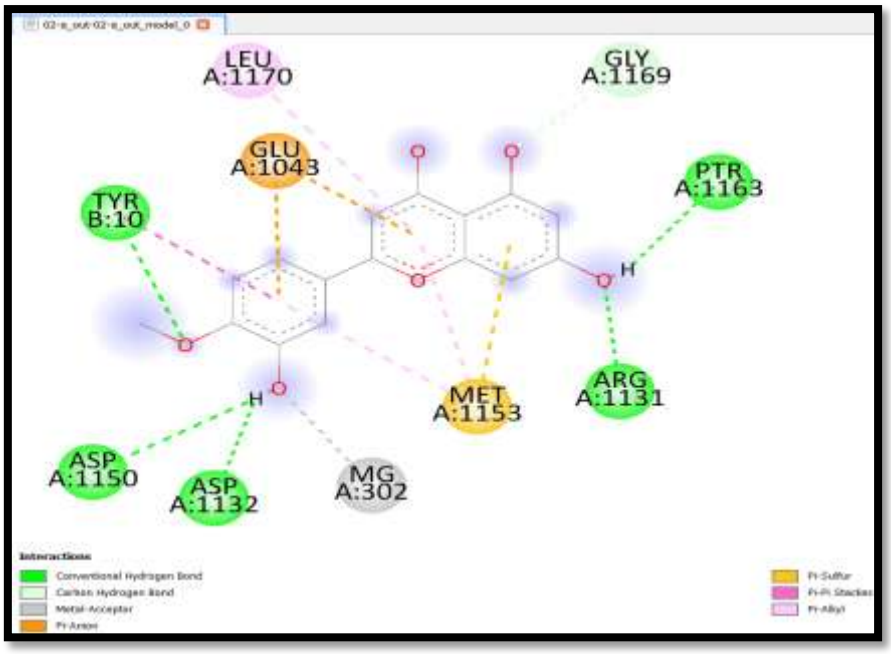
Compounds	Binding Energy (Kcal/mol)	No of H-bonds	H-bond Lengths (A0)	H-Bond Interactions	Interacting Amino Acids
EAME-01	-11.87	3	2.102, 2.564, 2.983	ARG:1131, ASP:1150, TYR:10	PTR:1163, MET:1153, GLU:1043, GLY:1169, TYR:10, MG: 302
MAME-02	-10.92	5	2.172, 2.19, 2.68, 2.54, 2.05	TYR:10, ASP:1150, ASP:1132, PTR:1163, ARG:1131	GLU:1043, EU:1170, GLY:1163, MET:1153, MG:302



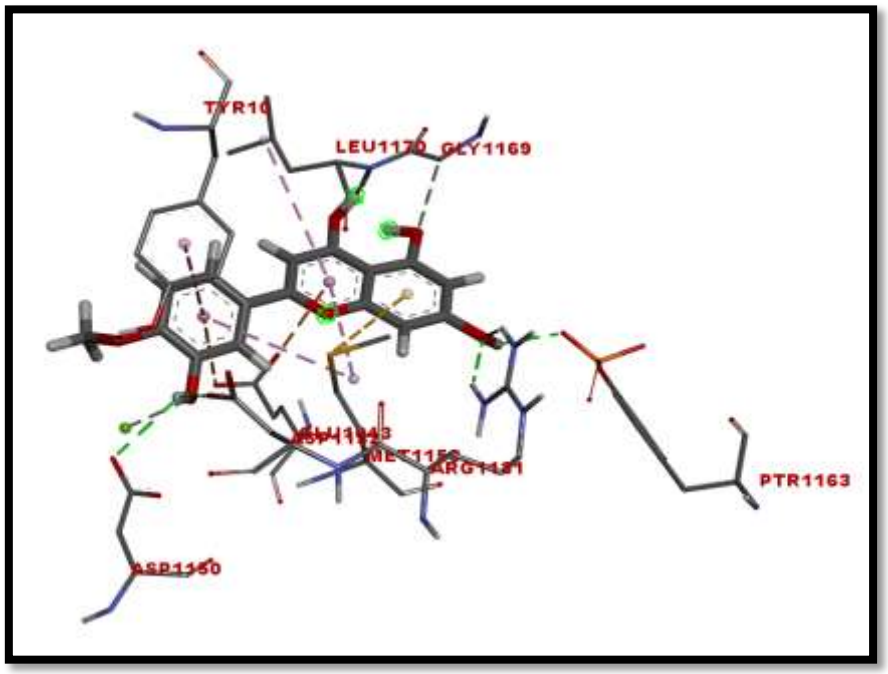
2D- EAME-01



3D- EAME-01



2D-MAME-02



3D-MAME-02

Fig 6. Molecular docking studies of Isolated compounds EAME-01 and MAME-02

CONCLUSION

The present study evaluated the phytochemical composition, antidiabetic activity, and molecular interactions of ethanol and methanol root extracts of Aquilaria malaccensis. Phytochemical screening revealed the presence of flavonoids in both extracts, while the isolated compounds EAME-01 and MAME-02 were characterized using IR, 1H NMR, and mass spectrometry. In vitro assays demonstrated

concentration-dependent α-amylase and α-glucosidase inhibitory activities, with EAME-01 showing the highest activity. Molecular docking revealed a favorable binding energy of −11.87 kcal/mol against the insulin receptor (PDB ID: 1IR3), supported by hydrogen bonds and other stabilizing interactions. These findings suggest that EAME-01 may be a promising candidate for further antidiabetic research. However, additional structural validation, enzyme-based studies, toxicity evaluation, and in vivo investigations are necessary to confirm its therapeutic potential.

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