Phytochemical Profiling and Evaluation of Antioxidant, Antidiabetic Potential of Aegle Marmelos Leaves

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ABSTRACT

Medicinal plants are an important source of natural bioactive compounds with significant therapeutic potential. *Aegle marmelos* is a well-known medicinal plant widely used in traditional systems of medicine for the treatment of various ailments, including diabetes and oxidative stress-related disorders. The present study was undertaken to evaluate the phytochemical constituents and biological activities of *Aegle marmelos* leaf extract.

Preliminary phytochemical screening was carried out using standard qualitative methods, which revealed the presence of important secondary metabolites such as flavonoids, glycosides and steroids. The total phenolic and total flavonoid contents of the extract were also determined, indicating the presence of polyphenolic compounds.

The antioxidant activity of the extract was evaluated using the DPPH free radical scavenging assay, while the antidiabetic potential was assessed through the α-amylase inhibition assay. The results demonstrated that the extract exhibits notable antioxidant and antidiabetic activities.

Further analysis using HPTLC confirmed the presence of flavonoid compounds, while LC–MS profiling revealed a diverse range of phytoconstituents, indicating the chemical complexity of the extract.

Overall, the findings suggest that *Aegle marmelos* leaves are a promising source of bioactive compounds with potential therapeutic applications, supporting their traditional medicinal use.

Keywords: Aegle marmelos, medicinal plants, phytochemical screening, total phenolic content, antioxidant activity, antidiabetic activity.

INTRODUCTION

Medicinal plants have been used for centuries in different traditional systems of medicine for the prevention and treatment of many diseases. They are considered an important source of natural bioactive compounds with significant therapeutic potential. These plants contain various secondary metabolites such as phenols, flavonoids, alkaloids and tannins which are responsible for several pharmacological activities. In recent years, plant-based medicines have gained considerable attention because they are relatively safer and easily accessible compared to synthetic drugs (Manzano et al.,2020).

Aegle marmelos, commonly known as Bael belongs to the family Rutaceae and is widely distributed in India. The plant has been widely used in traditional medicine and different parts of the plant such as leaves, fruits and bark are known to possess medicinal properties. Traditionally, the plant has been used in the treatment of digestive disorders, diabetes, inflammation and microbial infections. Because of its wide medicinal importance, Aegle marmelos has been extensively studied for its phytochemical constituents and pharmacological activities (Swarnkar et al., 2019). Phytochemical studies have reported that the plant is rich in bioactive constituents such as flavonoids, alkaloids, tannins, saponins, phenolic compounds. These bioactive compounds are responsible for its medicinal actions (Kokate et al. 2004).

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Diabetes mellitus commonly known as diabetes is a chronic metabolic disease in which there is an increase in blood glucose level in the body. This increase in blood glucose levels is known as hyperglycemia. It occurs due to insulin deficiency, Insulin resistance or both. If the disease is not treated properly it may result into the prolonged complications like kidney failure, heart diseases, foot ulcers, damage to the eyes, weight gain, hypoglycemia, gastrointestinal disturbances, liver toxicity. Diabetes can be characterized by abnormal metabolism of carbohydrates, lipids, lipoproteins. It is one of the most common disorders emerging worldwide (Credo et. al. 2018).

Phenolic compounds and flavonoids are among the major phytochemicals present in many medicinal plants. These compounds are well known for their antioxidant properties and their ability to neutralize free radicals. By reducing oxidative stress, they help in protecting biological systems from cellular damage. In addition to antioxidant activity, phenols and flavonoids are also reported to possess several biological activities such as anti-inflammatory, antimicrobial and antidiabetic effects (Pietta, 2000).

Therefore, the present study was carried out to evaluate the phytochemical constituents of *Aegle marmelos* leaves extract. The study also aimed to determine the total phenolic content and total flavonoid content of the extract. Furthermore, antioxidant and antidiabetic activities were investigated in order to understand the therapeutic potential of the plant.

MATERIALS AND METHODS

Collection of Plant Material

Fig.2.1 – Collection of Aegle marmelos leaves

Fresh leaves of Aegle marmelos were collected from the area of Hivra Khurd, Taluka Basmath, District Hingoli (Maharashtra, India). The site was surveyed during active growing season and only healthy plant material were selected for further study. The collected leaves were washed with water, shade dried and powdered using a mechanical grinder**.**

Preparation of Leaf Extract

The leaf extract was prepared by Soxhlet extraction method in the botany research laboratory, N. E. S. Science college, Nanded. The crushed leaf material of 5 gm was loaded into the thimble, which is placed inside the Soxhlet extractor. The thimble was filled with 300ml of Methanol extraction. The side arm was lagged with glass wool. The solvent was heated using the mantle and the solvent were begun to evaporate, moving through the

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apparatus to the condenser. The condensate then drips into the reservoir containing the thimble. Once the level of solvent reaches the siphon it pours back into the flask and the cycle begins again. The process was run for a total of 8 hours. After the successful extraction of 7 cycles the extracted plant sample were air dried and collected into the extraction collector for further use (Redfern et al.,2014; Kasiramar et al., 2019)

After the preparation of extract preliminary phytochemical analysis, estimation of total phenol content, estimation of total flavonoid content was also performed in the botany research laboratory, N.E.S. Science College, Nanded.

Preliminary Phytochemical Screening:

The preliminary phytochemical analysis tests are the simple qualitative tests helps to identify the major groups of phytochemicals present in the plant extract before performing the advanced techniques.

Preliminary phytochemical screening of the *Aegle marmelos* leaf extract was carried out using standard qualitative methods to detect the presence of different classes of secondary metabolites. Glycosides were identified using Keller–Killiani, Raymond's and Legal's tests. Alkaloids were detected by Mayer's test, while flavonoids were confirmed using ferric chloride, Shinoda and zinc hydrochloride reduction tests.

Steroids were determined by chloroform and Salkowski tests. Saponins were detected by the foam test. Carbohydrates were identified using Molisch's and Benedict's tests, while proteins were detected using Millon's, xanthoproteic, biuret and ninhydrin tests. Starch was confirmed by starch reagent test. Tannins were detected using gelatin and sodium hydroxide tests. The appearance of characteristic colour changes or precipitates indicated the presence of the respective phytochemical constituents (Harborne et al., 1998; Shaikh et al., 2020; Auwal et al., 2014).

Determination of Total Phenolic Content:

Total phenolic content of the extract was determined using the Folin–Ciocalteu reagent method. Different concentrations of the plant extract (20–100 µg/ml) were prepared. An aliquot of the extract was mixed with distilled water followed by the addition of Folin–Ciocalteu reagent. After 5 minutes, sodium carbonate solution was added to the mixture and the final volume was adjusted with distilled water.

Similarly, standard solutions of gallic acid were prepared for the calibration curve. The reaction mixture was incubated at room temperature and the absorbance was measured using a spectrophotometer at 550 nm against a reagent blank. The total phenolic content was expressed as µg of gallic acid equivalent (GAE) per g of extract, and all measurements were performed in triplicate (Chang et al., 2002; Phuyal et al.,2020; Ulloa et al., 2020).

Determination of Total Flavonoid Content

Total flavonoid content of the extract was determined using the aluminium chloride colorimetric method. The reaction mixture consisted of plant extract and distilled water followed by the addition of sodium nitrite solution. After incubation, aluminium chloride solution was added to the mixture and subsequently sodium hydroxide solution was introduced. The final volume was adjusted with distilled water.

Standard solutions of quercetin at different concentrations were prepared to obtain the calibration curve. The absorbance of both test and standard solutions was measured using a spectrophotometer at 510 nm against the reagent blank. The total flavonoid content was expressed as µg of quercetin equivalent (QE) per g of extract, and the analysis was performed in triplicate (Ashok et al., 2017; Kalita, et al.,2013; Bag et al., 2015).

Further assays such as antioxidant assay, antidiabetic assay was performed in the infinite biotech lab Sangli.

Antioxidant Activity:

The antioxidant activity of the plant extract was evaluated using the DPPH free radical scavenging assay. Standard used in this method is ascorbic acid.

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Different concentrations of the extract (20–100 µg/ml) were prepared. An aliquot of the extract was mixed with methanolic DPPH solution and the reaction mixture was incubated in the dark for about 30 minutes.

The change in colour of the solution from purple to yellow indicated the scavenging of DPPH free radicals. The absorbance of the reaction mixture was measured using a spectrophotometer at 510 nm against the control. The percentage of DPPH radical scavenging activity was calculated using the following equation:

DPPH scavenging activity (%) = [(Absorbance of control − Absorbance of sample) / Absorbance of control] × 100 (Baliyan et al., 2022; Silva et al., 2024; Jahan et al., 2024).

Antidiabetic Activity:

The antidiabetic activity of the plant extract was evaluated by in vitro α-amylase inhibition assay following the method of Bernfeld. Standard acarbose is used in this assay.

Different concentrations of the extract were prepared and mixed with phosphate buffer (pH 6.9) containing α-amylase enzyme. The reaction mixture was incubated for a short period at room temperature.

After incubation, starch solution was added as a substrate and the mixture was further incubated. The reaction was terminated by adding dinitro salicylic acid (DNSA) reagent and the mixture was heated in a boiling water bath. After cooling, the absorbance was measured using a spectrophotometer at 540 nm.

The percentage inhibition of α-amylase activity was calculated using the following equation:

Inhibition (%) = [(Absorbance of control − Absorbance of sample) / Absorbance of control] × 100

(Zephy et al., 2015; Maritim et al., 2003)

HPTLC Analysis

The HPTLC analysis of *Aegle marmelos* leaf extract was carried out using a systematic and optimized procedure. For sample preparation, an accurately weighed quantity of the extract was dissolved in distilled water, sonicated to ensure complete dissolution, and further diluted with methanol to obtain a final concentration of 50 ppm. A similar procedure was followed for the preparation of the standard solution using quercetin. Chromatographic separation was performed on pre-coated silica gel 60 F_{254} plates. The sample and standard solutions were applied as bands using an automated applicator. The plates were developed in a saturated chamber containing a mobile phase of toluene, ethyl acetate and formic acid in the ratio of 6:3:1 (v/v/v). After development up to an appropriate distance, the plates were dried and scanned at 329 nm using a densitometric scanner. The chromatograms were recorded and the Rf values of the sample were compared with those of the standard to identify the presence of phytoconstituents. This method allowed effective separation and characterization of bioactive compounds present in *Aegle marmelos* leaf extract (Thomas et al., 2020; Chewchinda et al., 2020; Sujatha et al., 2019).

LC-MS analysis

The LC–MS analysis of *Aegle marmelos* leaf extract was performed to identify and characterize the phytochemical constituents present in the sample. The dried and powdered plant material was extracted using a suitable solvent, and the obtained extract was filtered and concentrated. A portion of the extract was reconstituted in methanol to prepare a clear sample solution, which was further filtered through a membrane filter prior to injection into the LC–MS system.

Chromatographic separation was achieved using a liquid chromatography system equipped with a suitable analytical column under controlled conditions. The mobile phase consisted of an aqueous component and an organic solvent, which were delivered in a gradient elution mode to facilitate effective separation of compounds based on their polarity. The flow rate, injection volume and run time were maintained as per optimized conditions to obtain well-resolved chromatographic peaks.

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Mass spectrometric detection was carried out using a high-resolution QTOF mass spectrometer operating in positive ionization mode. The ion source parameters, including capillary voltage, drying gas temperature and nebulizer pressure, were adjusted to ensure efficient ionization of analytes. The mass spectra were acquired over an appropriate m/z range to detect a wide variety of phytoconstituents present in the extract.

The obtained chromatograms displayed multiple peaks corresponding to different compounds, each characterized by specific retention times and mass-to-charge ratios. Data acquisition and processing were performed using specialized software, and compound identification was carried out by comparing the obtained mass spectra with standard library databases. The compounds were tentatively identified based on their molecular mass, fragmentation pattern and matching score, ensuring reliable phytochemical profiling of *Aegle marmelos* leaf extract (Gandu et al., 2025; Thorsteinsdottir et al., 2021; Jouaneh et al., 2022).

RESULTS AND DISCUSSION

Preliminary Phytochemical Analysis

Fig. 3.1 - Preliminary Phytochemical Analysis of Aegle marmelos

Test for Glycosides	Result
1. Kellar Killani test	**Positive**
2. Raymond's test	Negative
3. Legal's test	**Positive**

Test for Alkaloids	
1.Mayer's test	Negative
Test for Flavonoids	
1. Ferric chloride test	**Positive**
2. Shinoda test	**Positive**
3. Zinc hydrochloric acid reduction test	Negative
4. Alkaline reagent test	Negative
5. Lead acetate solution test	**Positive**
Test for Steroids	
1. Chloroform test	**Positive**
2. Sakowaski test	**Positive**
Test for Phenols	
1. Ferric chloride test	Negative
Test for Saponins	
2. Foam test	Negative
Test for Carbohydrates	
1. Molisch's test	Negative
2. Benedict's test	**Positive**
Test for Proteins	
1. Millon's test	**Positive**
2. Xanthoproteic test	Negative
3. Biuret test	**Positive**
4. Ninhydrin test	Negative
Test for Starch	
1. Starch reagent test	Negative
Test for Tannins	
1. Gelatin test	Negative
2. NaOH test	Negative

Table 3.1: Qualitative phytochemical screening of *Aegle marmelos* leaf extract

Preliminary phytochemical screening of *Aegle marmelos* leaf extract showed the presence of glycosides, flavonoids, steroids, carbohydrates and proteins. Glycosides were confirmed by positive results in Keller–Killani and Legal's tests, while Raymond's test was negative. Alkaloids were not detected as Mayer's test showed a negative result.

Flavonoids were present as indicated by positive results in ferric chloride, Shinoda and lead acetate tests, whereas zinc hydrochloride reduction and alkaline reagent tests were negative. Steroids were confirmed by positive results in chloroform and Salkowski tests.

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Carbohydrates were detected by a positive Benedict's test, while Molisch's test was negative. Proteins were confirmed by positive Millon's and Biuret tests, whereas xanthoproteic and ninhydrin tests were negative.

Phenols, saponins, starch and tannins were not detected, as all respective tests showed negative results.

The present study demonstrates that *Aegle marmelos* leaf extract contains a variety of bioactive compounds that contribute to its biological activities. The preliminary phytochemical analysis confirmed the presence of flavonoids, glycosides, steroids, and proteins, indicating that the extract possesses significant pharmacological potential. These classes of compounds are widely known for their therapeutic properties, which supports the traditional use of the plant (Sivaraj et al., 2011)

Total Phenolic Content

Fig. 3.2 - Estimation of Total Phenolic Content by FCR method

The standard gallic acid solutions in the concentration range of 20–100 µg/ml showed a gradual increase in absorbance values from 0.102 to 0.520 at 550 nm, indicating a linear relationship between concentration and absorbance.

Similarly, the plant extract was analysed at different concentrations and the absorbance values were recorded in triplicate. The mean absorbance values increased with increasing concentration of the extract. Based on the calibration curve of gallic acid, the total phenolic content of the extract was calculated and expressed as µg gallic acid equivalent per gram (µg GAE/g) of extract.

The results showed that the phenolic content increased from 7.9 to 41.6 µg GAE/g with increasing concentration, indicating the presence of phenolic compounds in the extract**.**

Sr. No.	Concentration (µg/ml)	Test 1	Test 2	Test 3	Mean ± SD	Total Phenol Content (µg GAE/g)
1	20	0.078	0.080	0.081	0.079 ± 0.002	7.9
2	40	0.112	0.115	0.116	0.114 ± 0.002	11.4
3	60	0.252	0.254	0.256	0.254 ± 0.002	25.4
4	80	0.369	0.360	0.365	0.364 ± 0.004	36.4
5	100	0.420	0.412	0.418	0.416 ± 0.004	41.6

Table 3.2 Total phenolic content of *Aegle marmelos* leaf extract (Mean ± SD, n = 3)

Polyphenolic compounds are known to neutralize free radicals and reduce oxidative stress. Similar findings have been reported in *Aegle marmelos* and other medicinal plants (Rahman et al., 2024), supporting the biological significance of phenolic compounds.

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Total Flavonoid Content

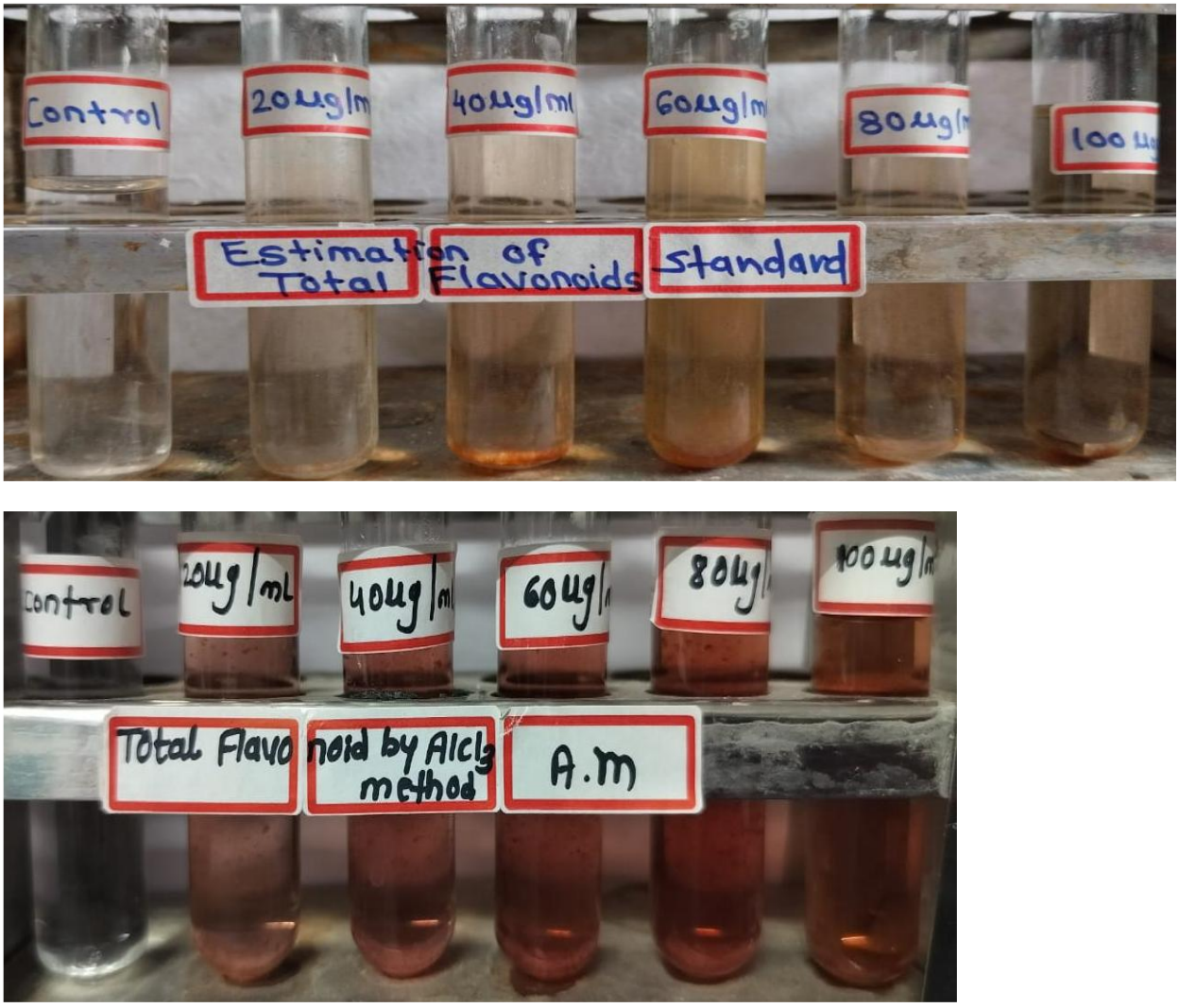

Fig. 3.3 – Total Flavonoid Content by Aluminium Chloride method

The standard quercetin solutions in the concentration range of 20–100 µg/ml showed a gradual increase in absorbance values from 0.343 to 0.677 at 510 nm, indicating a linear relationship between concentration and absorbance.

Similarly, the plant extract was analysed at different concentrations and the absorbance values were recorded in triplicate. The mean absorbance values increased with increasing concentration of the extract. Based on the calibration curve of quercetin, the total flavonoid content of the extract was calculated and expressed as µg quercetin equivalent per gram (µg QE/g) of extract.

The results showed that the flavonoid content increased from 7.9 to 41.6 µg QE/g with increasing concentration, indicating the presence of flavonoids in the plant extract.The results indicated the presence of flavonoids in the plant extract, which may contribute to its biological activities.

Sr. No.	Concentration (µg/ml)	Test 1	Test 2	Test 3	Mean ± SD	Total Flavonoid Content (µg QE/g)
1	20	0.112	0.115	0.119	0.115 ± 0.004	11.5
2	40	0.231	0.235	0.239	0.235 ± 0.004	23.5
3	60	0.315	0.319	0.322	0.318 ± 0.004	31.8
4	80	0.447	0.450	0.455	0.450 ± 0.004	45.0
5	100	0.572	0.575	0.579	0.575 ± 0.004	57.5

Table 3.3 Total flavonoid content of *Aegle marmelos* leaf extract (Mean ± SD, n = 3)

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Flavonoids are known for their antioxidant potential, as they scavenge free radicals and reduce oxidative stress. The observed results suggest that flavonoids may contribute to the biological activities of the extract. Similar findings have been reported in *Aegle marmelos* (Vardhini et al.,2018).

Antioxidant assay

The results showed that the percentage inhibition increased with increasing concentration of the extract. The standard ascorbic acid exhibited percentage inhibition ranging from 26.42% to 76.16% at concentrations of 20–100 µg/ml, with an IC_{50} value of 58.30 µg/ml.

Similarly, the Aegle marmelos extract showed concentration-dependent antioxidant activity with percentage inhibition ranging from 12.43% to 59.06% at concentrations of 20–100 µg/ml. The IC_{50} value of the extract was found to be 86.32 µg/ml. These results indicate that the plant extract possesses considerable antioxidant activity.

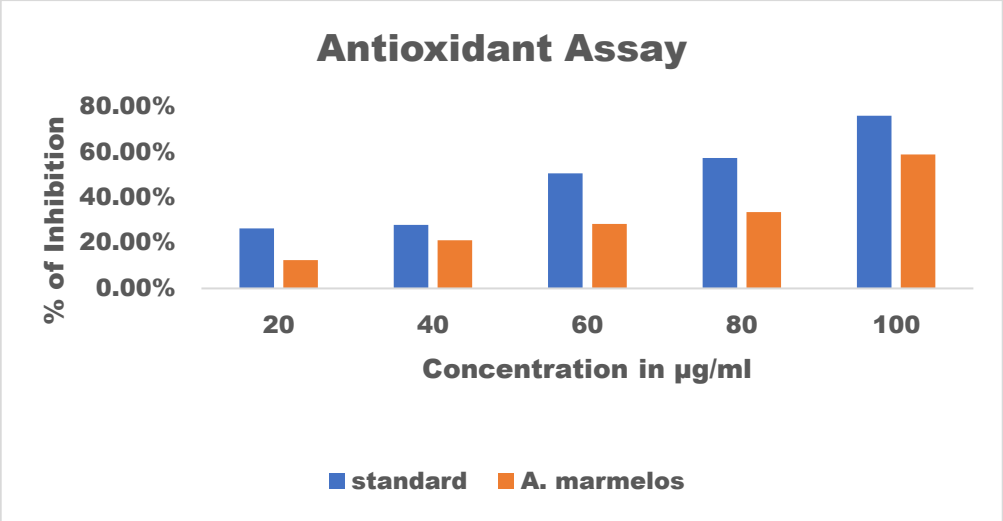

Fig. 3.4 Graph showing concentration dependent increase in antioxidant activity

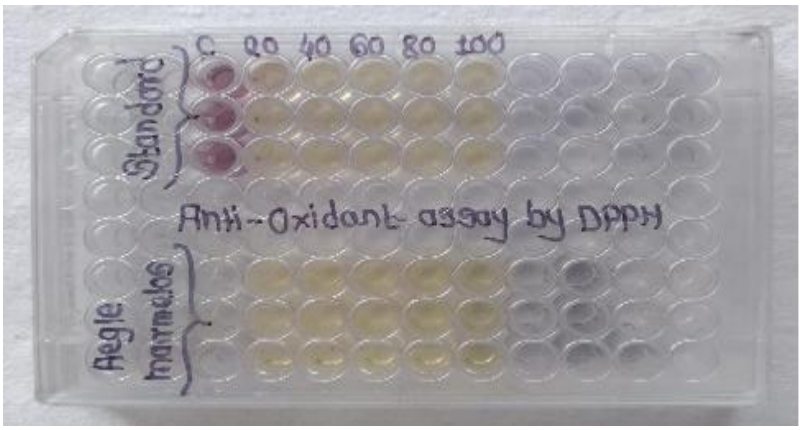

Fig. 3.5 - 96 wells ELISA plate showing colour change from purple to yellow at different concentrations

Standard (Ascorbic Acid)

Sr. No.	Concentration (µg/ml)	Test 1	Test 2	Test 3	Mean ± SD	% Inhibition
1	20	1.45	1.42	1.40	1.42 ± 0.02	26.42%
2	40	1.39	1.40	1.38	1.39 ± 0.01	27.97%
3	60	0.95	0.97	0.93	0.95 ± 0.02	50.77%
4	80	0.82	0.85	0.79	0.82 ± 0.03	57.51%
5	100	0.46	0.45	0.46	0.46 ± 0.01	76.16%

Table 3.4: DPPH free radical scavenging activity of standard ascorbic acid (Mean ± SD, n = 3)

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The observed antioxidant potential of the extract may be attributed to the presence of phenolic and flavonoid compounds, which are capable of donating electrons or hydrogen atoms to neutralize free radicals. These compounds play an important role in reducing oxidative stress and protecting biological systems from cellular damage. The activity also suggests the involvement of multiple bioactive constituents acting synergistically. Similar studies have been reported in *Aegle marmelos* and other plants (Reddy et al., 2013).

Antidiabetic assay

The results showed that the percentage inhibition increased with increasing concentration of the extract.

The standard drug acarbose exhibited percentage inhibition ranging from 6.89% to 75.86% at concentrations of 20–100 µg/ml, with an IC_{50} value of 62.13 µg/ml.

Similarly, the Aegle marmelos extract showed concentration-dependent inhibition of the α-amylase enzyme with percentage inhibition ranging from 12.06% to 55.74% at concentrations of 20–100 µg/ml. The IC_{50} value of the extract was found to be 94.26 µg/ml, indicating its potential antidiabetic activity.

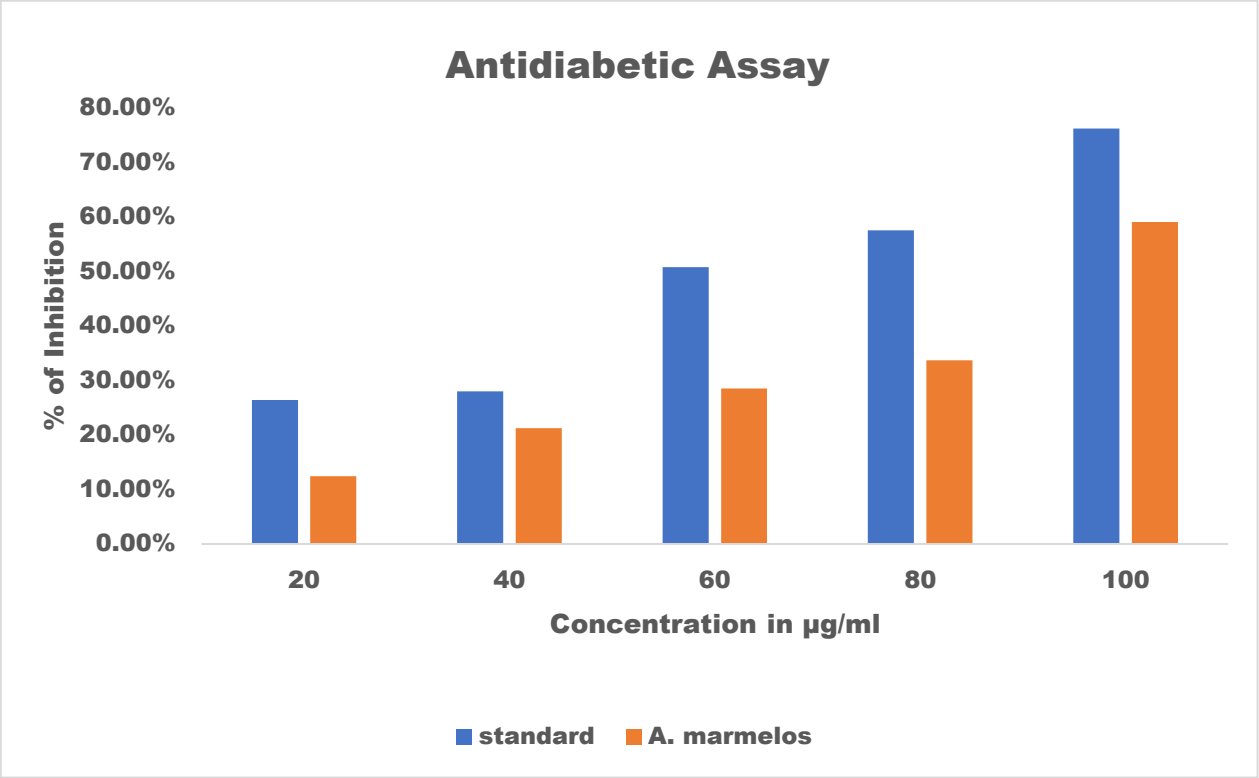

Fig. 3.6 - Graph showing dose dependent increase in antidiabetic activity

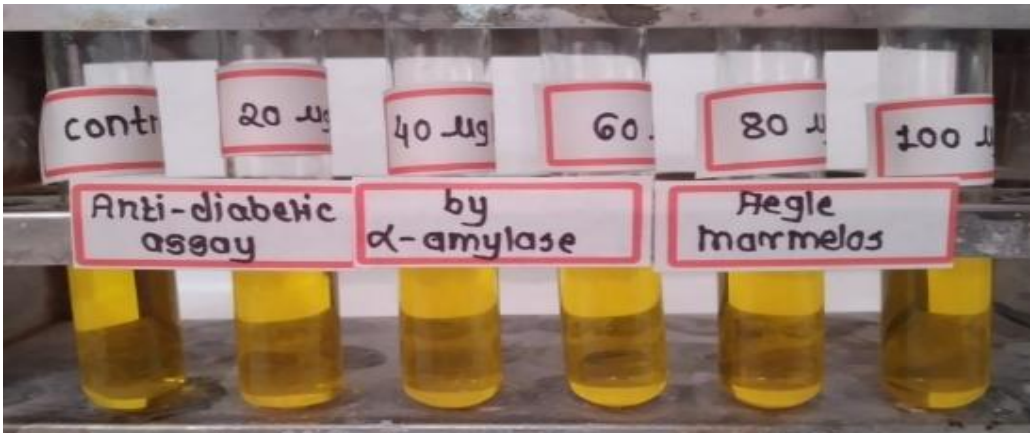

Fig.3.7 - α-amylase inhibition assay showing antidiabetic activity of *Aegle marmelos* extract at different concentrations

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Standard Acarbose

Sr. No.	Concentration (µg/ml)	Test 1	Test 2	Test 3	Mean ± SD	% Inhibition
1	20	1.62	1.62	1.63	1.62 ± 0.01	6.89%
2	40	1.31	1.32	1.31	1.31 ± 0.01	24.71%
3	60	1.09	1.08	1.07	1.08 ± 0.01	37.93%
4	80	0.82	0.83	0.82	0.82 ± 0.01	52.87%
5	100	0.43	0.43	0.42	0.42 ± 0.01	75.86%

Table 3.5: α-amylase inhibitory activity of standard acarbose (Mean ± SD, n = 3)

Aegle marmelos

Sr. No.	Concentration (µg/ml)	Test 1	Test 2	Test 3	Mean ± SD	% Inhibition
1	20	1.53	1.56	1.51	1.53 ± 0.02	12.06%
2	40	1.32	1.29	1.35	1.32 ± 0.02	24.13%
3	60	1.21	1.19	1.24	1.21 ± 0.02	30.45%
4	80	1.07	1.09	1.05	1.07 ± 0.02	38.50%
5	100	0.78	0.76	0.79	0.77 ± 0.02	55.74%

Table 3.6: α-amylase inhibitory activity of *Aegle marmelos* leaf extract (Mean ± SD, n = 3)

The inhibitory effect on α-amylase activity may be attributed to the presence of bioactive compounds such as flavonoids and polyphenols, which are known to interfere with carbohydrate metabolizing enzymes. These compounds can reduce glucose release by slowing down the breakdown of starch, thereby helping in the management of postprandial hyperglycaemia. The activity also indicates the potential of the extract as a natural alternative to synthetic antidiabetic agents. Similar mechanisms have been reported in medicinal plants, including *Aegle marmelos* (Venkatesan et al., 2024).

All experiments such as total phenols, total flavonoids, antioxidant assay and antidiabetic assay were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). The low variation among replicate values indicates good precision and reliability of the experimental data.

HPTLC analysis

The HPTLC analysis of *Aegle marmelos* leaf extract revealed a well-resolved chromatographic profile under the selected experimental conditions. The standard quercetin produced a distinct peak at an Rf value around 0.48, confirming the suitability of the chromatographic system. The leaf extract of *Aegle marmelos* exhibited a prominent peak at an Rf value of 0.52, which is in close agreement with the standard, indicating the presence of flavonoid constituents in the sample.

The densitometric scan recorded at 329 nm showed a sharp and symmetrical peak with good intensity, suggesting effective separation of the major phytoconstituent. The consistency of the peak position and shape indicates the reliability of the analytical method. The presence of a major peak in the region corresponding to quercetin suggests that *Aegle marmelos* leaves contain flavonoid compounds that may contribute to their antioxidant potential.

Overall, the HPTLC profile confirms that *Aegle marmelos* leaf extract possesses identifiable flavonoid components, supporting the results obtained from flavonoid content estimation and other biological assays.

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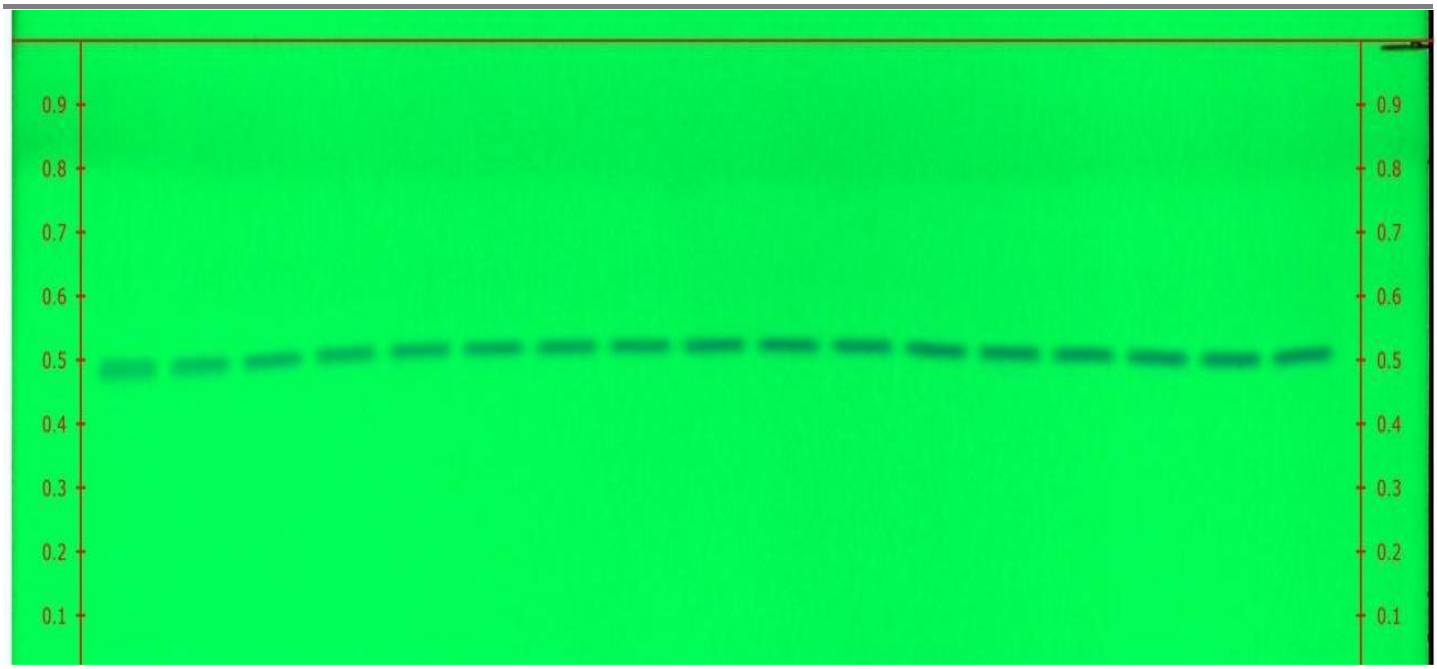

Fig 3.8 -HPTLC chromatogram of *Aegle marmelos* leaf extract and quercetin standard under UV 254 nm

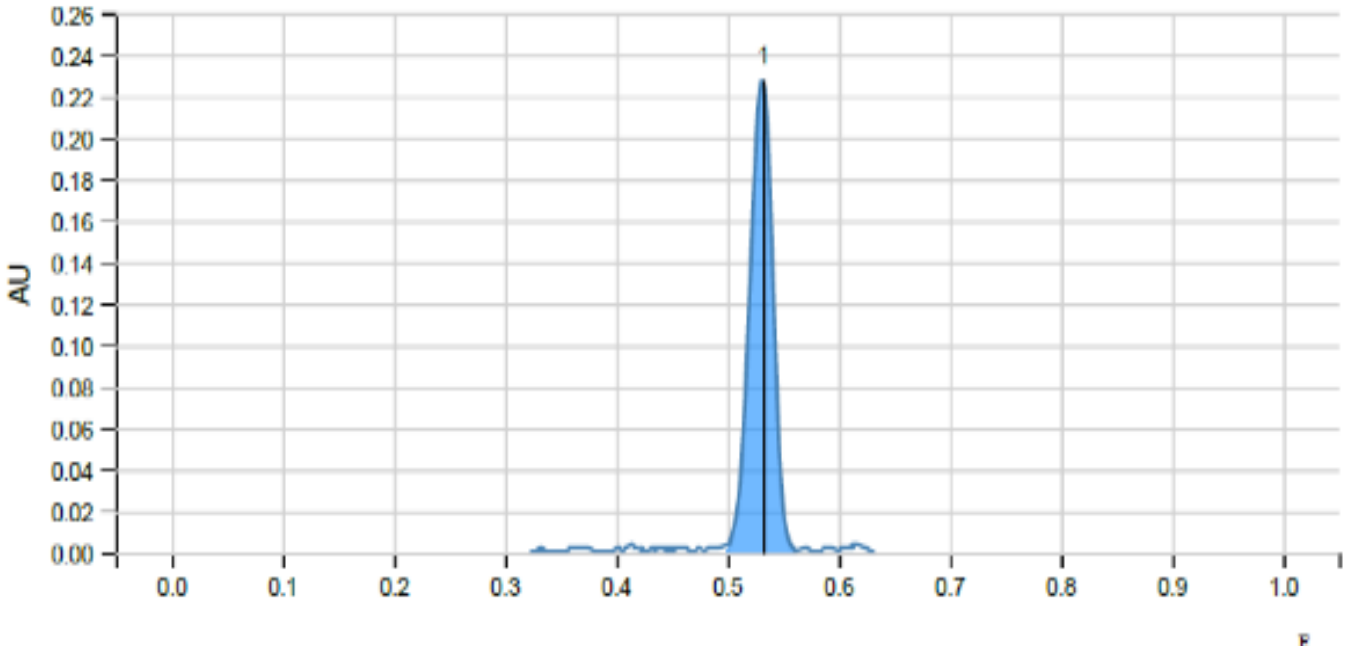

Fig. 3.9 - HPTLC densitogram of *Aegle marmelos* leaf extract showing a prominent peak at Rf 0.52, indicating the presence of flavonoid compounds.

The chromatographic profile suggests the presence of flavonoid constituents in the extract, as indicated by the similarity in migration pattern with the standard compound. The consistent peak characteristics reflect good separation and reliability of the analytical method. The detection of flavonoid-related bands supports the phytochemical findings and indicates their possible role in the observed biological activities (Rathod et al., 2024).

LC-MS analysis

The LC–MS analysis of *Aegle marmelos* leaf extract resulted in the tentative identification of 57 compounds, indicating the complex phytochemical nature of the extract. The major bioactive compounds identified and summarized in the table belong predominantly to flavonoid and polyphenolic classes.

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Tiliroside was detected at a retention time of 8.247 min, while 7-deoxyloganate was observed at 9.073 min. The presence of phenolic compounds was confirmed by the detection of (-)-epicatechin at 10.162 min.

Flavonoid glycosides such as myricetin 3-arabinoside (12.187 min), quercetin (12.741 min), and kaempferol 7-O-glucoside (13.467 min) were identified, indicating the abundance of flavonoid constituents in the extract. Additional flavonoids including guajavarin (13.249 min), diosmetin glycoside (14.193 min), and afzelin (14.259 min) were also detected.

Furthermore, the presence of anthocyanin compounds was indicated by the detection of cyanidin derivative at 15.015 min. The identification of these compounds confirms that the extract contains a diverse range of bioactive phytoconstituents.

Overall, the LC–MS results demonstrate that *Aegle marmelos* leaf extract is rich in flavonoids, phenolics and glycosidic compounds, which may contribute to its observed antioxidant and antidiabetic activities.

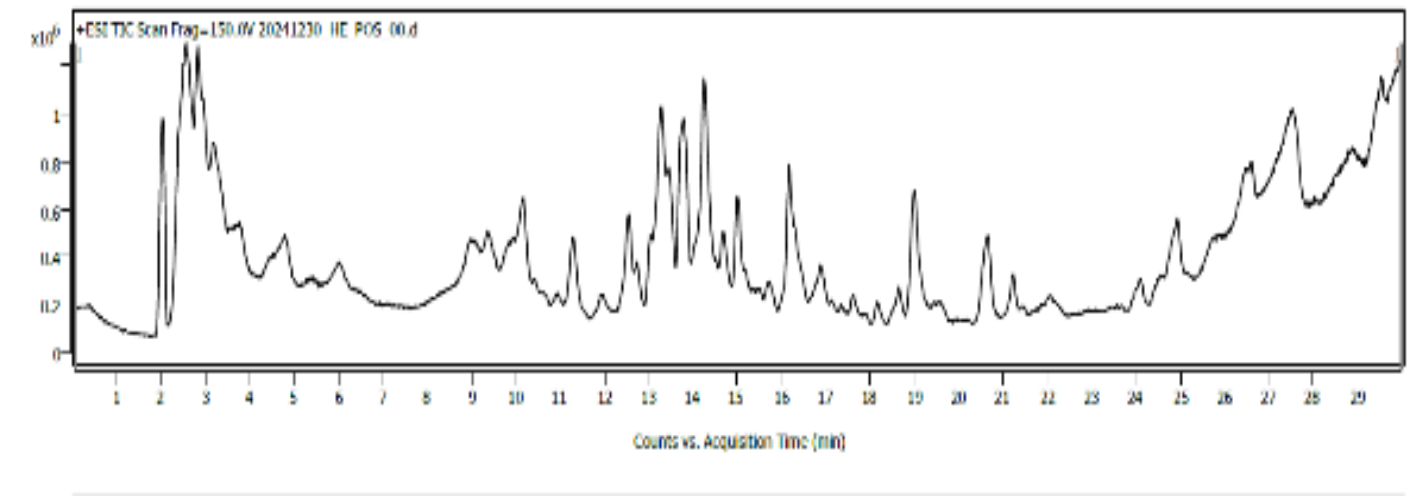

Fig.3.10- LC–MS total ion chromatogram of *Aegle marmelos* leaf extract showing multiple peaks corresponding to various phytoconstituents.

Sr. No	Retention Time (min)	Molecular Formula	Molecular Weight (g/mol)	Compound Name	Class	Phytofamily
1	8.247	$C_{30}H_{26}O_{13}$	594.1363	Tiliroside	Flavonoid	Malvaceae
2	9.073	$C_{16}H_{24}O_9$	360.1416	7-Deoxyloganate	Polyphenol	Amaryllidaceae
3	10.162	$C_{15}H_{14}O_6$	290.0792	(-)-Epicatechin	Phenolic	Theaceae
4	12.187	$C_{20}H_{18}O_{12}$	450.0794	Myricetin 3-arabinoside	Flavonoid	Theaceae
5	12.741	$C_{15}H_{10}O_7$	302.0423	Quercetin	Flavonoid	Rutaceae
6	13.249	$C_{20}H_{18}O_{11}$	434.0852	Guajavarin	Flavonoid	Myrtaceae
7	13.467	$C_{21}H_{20}O_{11}$	448.1007	Kaempferol 7-O-glucoside	Flavonoid	Liliaceae
8	14.193	$C_{22}H_{20}O_{12}$	476.0952	Diosmetin 7-O-beta-D-glucuronopyranoside	Flavonoid	Rutaceae
9	14.259	$C_{21}H_{20}O_{10}$	432.1054	Afzelin	Flavonoid	Rosaceae
10	15.015	$C_{23}H_{23}O_{12}$	491.1193	Cyanidin 3-(6"-acetyl- galactoside)	Anthocyanin	Nymphaeaceae

Table 3.7: Major phytoconstituents identified in *Aegle marmelos* leaf extract by LC–MS analysis

The identification of multiple phytoconstituents, particularly flavonoids and polyphenolic compounds, highlights the chemical complexity of the extract. These compounds are known for their significant antioxidant and antidiabetic properties, suggesting their contribution to the biological activities observed. The presence of

diverse bioactive molecules also indicates potential synergistic effects, which may enhance the overall therapeutic efficacy of the extract (Meena et al., 2022).

CONCLUSION

The present study provides a comprehensive evaluation of the phytochemical composition and pharmacological potential of *Aegle marmelos* leaf extract. Preliminary phytochemical screening confirmed the presence of important bioactive constituents such as flavonoids, glycosides, steroids and proteins, indicating the therapeutic relevance of the plant.

The quantitative estimation revealed appreciable amounts of total phenolic content (up to 41.6 µg GAE/g) and total flavonoid content (up to 57 µg QE/g), suggesting that the extract is rich in polyphenolic compounds. These findings were further supported by the biological assays, where the extract exhibited concentration-dependent antioxidant activity in the DPPH assay with an IC_{50} value of 86.32 µg/ml, and notable α-amylase inhibitory activity with an IC_{50} value of 94.26 µg/ml, indicating its potential antidiabetic effect.

The HPTLC analysis confirmed the presence of flavonoid compounds, showing a prominent peak at an Rf value of approximately 0.52, which was comparable to the standard quercetin. This indicates the presence of quercetin-like flavonoids and validates the phytochemical and flavonoid content results.

Further, LC–MS profiling revealed a complex chemical composition, with a total of 57 compounds tentatively identified. Among these, major bioactive compounds such as quercetin, kaempferol glycosides, myricetin derivatives, epicatechin, procyanidin B5, tiliroside, afzelin and guajavarin were detected. These compounds predominantly belong to flavonoid and polyphenolic classes and are well known for their antioxidant and antidiabetic properties.

Overall, the integration of phytochemical screening, quantitative estimation, chromatographic analysis and biological assays clearly demonstrates that *Aegle marmelos* leaves are a rich source of bioactive phytoconstituents with significant antioxidant and antidiabetic potential. The findings of this study provide scientific validation for the traditional use of the plant and suggest its potential for further pharmacological exploration and development of plant-based therapeutic agents

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