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IN SILICO AND PHARMACOLOGICAL EVALUATION OF ANTIDIABETIC AND ANTIOXIDANT POTENTIAL OF SECONDARY METABOLITES FROM AEGLE MARMELOS

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels due to impaired insulin secretion or insulin resistance. Oxidative stress is one of the major factors responsible for diabetic complications such as neuropathy, nephropathy, and cardiovascular disorders. Medicinal plants rich in phytoconstituents are widely explored as safer alternatives to synthetic drugs. *Aegle marmelos* (Bael) is an important medicinal plant traditionally used in Ayurveda for the treatment of diabetes and other disorders. The present study was aimed at evaluating the antidiabetic and antioxidant potential of secondary metabolites from *Aegle marmelos Linn.* through in silico and pharmacological approaches. Molecular docking studies were performed using selected phytoconstituents such as quercetin, rutin, aegeline, against diabetic target proteins including DPP-4, α-glucosidase, α-amylase. The docking analysis revealed that rutin showed the highest binding affinity against α-glucosidase and DPP-4, indicating strong inhibitory activity. The leaves of *Aegle marmelos* were collected, shade dried, powdered, and extracted using Soxhlet extraction. Preliminary phytochemical screening confirmed the presence of flavonoids, tannins, alkaloids, saponins, and cardiac glycosides. In vitro antidiabetic activity was evaluated by α-amylase inhibitory assay, while antioxidant activity was determined using FRAP assay. The extract demonstrated concentration-dependent antidiabetic and moderate antioxidant activity compared with standard drugs. The study concludes that *Aegle marmelos Linn.* possesses promising antidiabetic and antioxidant properties due to the presence of bioactive phytoconstituents. These findings support the traditional use of the plant and suggest its potential for the development of herbal therapeutic agents for diabetes management.

KEYWORDS: *Aegle marmelos Linn,* Diabetes mellitus, Antioxidant activity, Molecular docking, α-amylase inhibition, Phytochemicals, Rutin, Quercetin, In vitro evaluation, Medicinal plants.

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia. The flow in secretion and in diabetes, the activity of insulin results in hyperglycemia. Diabetes mellitus is the main threat to human health because of changes in lifestyle and behaviour, which have led to an increase in diabetes cases globally. Due to increased glycosylated proteins and glucose autooxidation, diabetics exhibited abnormal antioxidant status. Tissue damage is caused by oxidative stress, which also manifests as retinopathy, nephropathy, and coronary heart disease. In any case, lowering blood pressure, blood sugar, and cholesterol could lower the chance of cardiac issues. The biological system experiences oxidative stress during the metabolic process. Additionally, this stress has been linked to diabetes and the consequences it causes in people. Oxidative Enzyme inactivation, structural alterations in collagen, and protein glycation are all caused by stress.[1]

Nature provides a vast reservoir of cures for human ailments. Approximately 80% of people on the planet rely entirely or partially on traditional medicine to meet their basic medical needs. A World Health Organization report states that approximately 80% of patients in India, 85% in Burma, and 90% in Bangladesh are treated by conventional medical professionals. For thousands of years, traditional medical systems have included herbal remedies, which have greatly improved health. Secondary metabolites and essential oils with therapeutic value are abundant in medicinal plants. In addition to being affordable, efficient, and readily accessible, the main benefits of using medicinal plants for therapeutic purposes in a variety of illnesses are their safety.[2]

People search for alternative medical systems due to the failure of current medicine, adverse drug reactions, and the startlingly high expense of allopathy treatments. This covers Ayurveda, Unani, Siddha, and medicinal plant therapy such as acupuncture. According to estimates, home care and traditional indigenous medical systems account for 80% of medical treatment in the current day. A significant portion of these therapies use plant extracts or their active ingredients. Effective chemotherapeutants are stored in angiosperm plants, and biological screening of these plants for a variety of activities revealed that they can be utilized to treat conditions like dyspepsia, diarrhoea, dysentery stomach discomfort. Many plant species have had their antibacterial activity assessed over the past 20 years. As a result, the scientific foundation for these plants' medicinal properties must be established. This study aims to assess the antimicrobial activity of A. marmelos leaves, bark, and fruits. The antibacterial activity of leaf, bark, and fruit extracts is evaluated in light of the traditional medical applications of A. marmelos.[3]

Phytochemicals useful for the development of new drugs may be found in medicinal plants. Phytochemicals with various therapeutic effects, including antimicrobial and antioxidant activity, include carotenoids, terpenoids, flavonoids, polyphenols, alkaloids, tannins, saponins, pigments, enzymes, minerals, and vitamins. The majority of contemporary medications come from natural sources, and many of these isolations were inspired by the agents' applications in conventional medicine. Medicinal plants have long been used to prevent and treat illnesses, and they are still essential for maintaining both human and animal health. The foundation of both the traditional medical system and the herbal business for the creation of new drugs is still medicinal plants. Alkaloids, cardiac glycoside, saponin, steroids, coumarines, terpenoids, phenylpropenoids, tannins, polysaccharides, and flavonoids are among the significant phytochemicals that have been extracted from different sections of the plant. The pharmacological and biological action of these phytochemical components against some chronic diseases, including as cancer, cardiovascular disease, immunosuppressive disease, and gastrointestinal disorders, is widely recognized. The plant extract components have anticonvulsant, antioxidant, antihyperglycemic, antibacterial, hepatoprotective, and antithyroid properties. Eighty percent of the world's population uses plant extracts or their active ingredients in traditional medicines, according to the World Health Organization. Over 50% of all modern clinical drugs are of natural product origin. In India, drugs of herbal preparations have been used in traditional system of medicines such as Unani and Ayurveda since ancient times. Three species in the tiny genus *Aegle marmelos Linn*. are found throughout tropical Asia and Africa. Originating in the Eastern Ghats and central India, the *A. marmelos Linn.* tree is mostly found in the foothills of the Himalayas, Uttar Pradesh, Madhya Pradesh, Rajasthan, Chhattisgarh, and Bihar.[4]

Bael, also known as *Aegle marmelos Linn.* (L.) Correa, is a deciduous subtropical tree in the Rutaceae family and the sole species in the genus Aegle. It is indigenous to the Indian subcontinent and widely distributed throughout Southeast Asia.[5]

It is valued for both its cultural importance in traditional medicinal systems and its resistance to arid environments. The hard shelled, mucilaginous pulp-filled. Bael fruit has been utilized as food and medicine for many generations.[6]

Bael is referred to in Indian Ayurvedic literature as a "divine tree," emphasizing its use in rituals, functional foods, and home remedies. Phytochemical study indicates that nearly every part of the tree, including the fruit, leaves, bark, roots, seeds, and flowers, contains a variety of bioactive substances, including coumarins, flavonoids, terpenoids, phenolic acids, and alkaloids. The wide spectrum of pharmacological effects linked to these metabolites, such as antibacterial, anti-inflammatory, antidiabetic, anticancer, and neuroprotective activity, supports its traditional claims. Despite its lengthy history of ethnomedical use and growing pharmacological data, bael's translation into modern phytopharmaceuticals is still limited. Although previous reviews often provide fragmented insights into either its phytochemistry or specific pharmacological qualities, a comprehensive synthesis that integrates mechanistic pathways, molecular docking evidence, clinical findings, and translational gaps is lacking. Variations in phytoconstituent profiles, irregular extraction methods, and the absence of standardized clinical studies further restrict its clinical utility.[7]

For thousands of years, people have used plants as a natural supply of therapeutic chemicals. Many plants and plant-derived items are used by humans as remedies for a variety of physical and mental ailments. Traditional Chinese, Ayurvedic, Siddha, Unani, and Tibetan remedies all contain these herbs. The use of plants to cure a variety of health issues is also described in ancient texts like the Rigveda, Yajurveda, Atharvaveda, Charak Samhita, and Sushrut Samhita. Over the past fifty years, these plants have been thoroughly investigated using cutting-edge scientific methods and reported for a variety of therapeutic qualities, including anticancer, antibacterial, antifungal, antidiabetic, antioxidant, hepatoprotective, hemolytic, larvicidal, and anti-inflammatory properties. Because of its ability to treat ailments like diabetes and related illnesses, *Aegle marmelos Linn*. is regarded as a food supplement in India and is listed in the Ayurvedic Pharmacopeia of India. While many medical systems, including Ayurveda, Unani, Chinese, and Tibetan medicine, revere and use medicinal plants for therapeutic purposes, most traditional medicines have not had their pharmacological usefulness tested or confirmed byexperimentation. Therefore, in order to establish the quality control of the alcoholic extract of *A. marmelos*, extensive and thorough phytochemical profiling is required. GC/MS and HPLC have become the preferred methods for phytochemical identification and are increasingly being used to characterize the structure of complex matrices. Furthermore, no studies on the anti- inflammatory, antioxidant, cytoprotective, and glucose-utilizing characteristics of *A. marmelos Linn.* have been published to far. Therefore, the purpose of this study was to investigate its phytoconstituents chromatographically. Additionally, we looked into *A. marmelos's* pharmacological, antioxidant, and toxicological potential.[8]

Bael, or *Aegle marmelos Linn*. (L.) Correa, is the only species in the genus Aegle and a deciduous subtropical tree in the Rutaceae family. It is extensively dispersed throughout Southeast Asia and is native to the Indian subcontinent. It is prized for its ability to withstand dry conditions and for its cultural significance in traditional medical systems. The hard-shelled, mucilaginous pulp-filled Bael fruit has been used for generations as a food and medicine in India. Ayurvedic writings refer to Bael as a "divine tree," highlighting its incorporation into ceremonies, functional foods, and home cures. Nearly every component of the tree, including the fruit, leaves, bark, roots, seeds, and flowers, contains a variety of classes of bioactive chemicals, including as coumarins, flavonoids, terpenoids, phenolic. Numerous kinds of bioactive compounds, such as coumarins, flavonoids, terpenoids, phenolic acids, and alkaloids, are found in almost every part of the tree, including the fruit, leaves, bark, roots, seeds, and flowers, according to phytochemical research. Its traditional claims are supported by the extensive range of pharmacological actions associated with these metabolites, including antibacterial, anti-inflammatory, antidiabetic, anticancer, and neuroprotective activity.[9]

Figure 1: *Aegle marmelos* plant leaves.[10]

Plant Profile

Table 1: Plant Profile.[11,12]

Biological source	Dried or fresh leaves, fruits and roots of *Aegle marmelos Linn.*
Family	Rutaceae
Common names	English: Bael Hindi: Bel Kannada: Bela Marathi: Bel
Geographical sources	India, Ceylon, China, Nepal, Sri Lanka, Myanmar, Pakistan, Bangladesh, Nepal, Vietnam, Laos, Cambodia, Thailand, Indonesia, Malaysia, Tibet, Sri Lanka, Java, Philippines and Fiji.
Biological description	Tree: Medium-sized tree with spines, 25 to 30 feet tall. Leaves: Trifoliate (3 leaflets), Aromatic smell, spherical or oval in shape with a diameter of 2 to 4 inch. Fruit: Hard outer shell, Yellow/orange pulp inside, pulp of the fruit has 8 to 15 segments. The pulp is yellow, soft, pasty, sweet, resinous and fragrant.
Chemical constituents	Alkaloids (e.g., aegeline) Flavonoids (quercetin, rutin) Tannins Coumarins
Traditional use	Jaundice, constipation, chronic diarrhea, dysentery, stomachache, stomachic, fever, asthma, inflammations, febrile delirium, acute bronchitis, snakebite, abdominal discomfort, acidity, burning sensation, epilepsy, indigestion, leporsy, myalgia, smallpox, spermatorrhoea, leucoderma, eye disorders, ulcers, mental illnesses, nausea, sores, swelling, thirst, thyroid disorders, tumors, ulcers and upper respiratory tract infections.

MATERIALS AND METHODS

➢ **Collection of plant material**

Fresh and healthy leaves of *Aegle marmelos Linn.* were collected from a suitable local area. The collected leaves were washed thoroughly with water to remove impurities and shade dried at room temperature. The dried leaves were powdered using a mechanical grinder and passed through sieve no. 60 to obtain uniform particle size.

➢ **Chemicals and Reagents**

The chemicals and reagents used in the study included ethanol, petroleum ether, distilled water, phosphate buffer, ferric chloride, potassium ferricyanide, trichloroacetic acid, α-amylase enzyme, starch solution, DNS reagent, ascorbic acid, and metformin.[13]

➢ **Apparatus**

1. Filter paper (Inert)

2. Glass Test tube
3. Beakers (50ml,100ml,250ml)
4. Conical flasks (50ml,100ml,250ml)
5. Sieve (No.60) Approx 250 microns
6. Mortar and pestle
7. Measuring Cylinder
8. Soxhlet Assembly

- Round bottom flask (contains the solvent)
- Thimble (Holds powder drug)
- Siphon tube
- Condenser

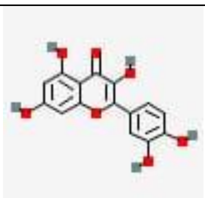
9. Heating mantle/ Water bath
10. Pipettes

➢ **Equipment**
1. Analytical balance
2. Incubator
3. UV visible spectrophotometer
4. Hot air oven

In silico study

(a) Selection of Phytoconstituents: The phytoconstituents of *Aegle marmelos Linn.* such as aegeline, quercetin, and rutin were selected based on reported pharmacological activities.

Table 2: Phytoconstituents Pubchem Id and structure.[14,15,16]

Sr. no.	Phytoconstituent	PubChem Id	Stucture
1	Quercetin	5280343	
2	Rutin	5280805	

3	Aegeline	15558471	

The structures were downloaded from "PubChem"

(b) Protein data

Table 3: Protein data and PDB Id.[17,18,19]

Sr. no.	Target protein	Function	PDB Id
1	DPP-4	Degrades incretin hormone (GLP-1, GIP)	5Y7H
2	Alpha glucosidase	Converts carbohydrates into glucose	7T6W
3	Alpha Amylase	Breaks down starch into sugars	1MWO

The protein structures were retrieved from "Protein data bank"

Table 4: Binding Affinity analysis.

Sr. no.	Phytoconstituents	Target protein	Binding affinity(kcal/mol)
1	Quercetin	DPP-4	-8.9
2	Quercetin	Alpha-glucosidase	-8.7
3	Quercetin	Alpha-amylase	-7.9
4	Rutin	DPP-4	-9.8
5	Rutin	Alpha-glucosidase	-10.2
6	Rutin	Alpha-amylase	-8.4
7	Aegeline	DPP-4	-7.3
8	Aegeline	Alpha-glucosidase	-8.1
9	Aegeline	Alpha-amylase	-7.2

The binding affinity results indicate that rutin exhibited the highest binding affinity, particularly against α-glucosidase (-10.2 kcal/mol), followed by strong interaction with DPP-4 (-9.8 kcal/mol). Quercetin also showed significant binding with DPP-4 and α-glucosidase. In contrast, aegeline demonstrated comparatively lower binding affinities. These results suggest that flavonoids like rutin and quercetin may contribute significantly to the antidiabetic potential of *Aegle marmelos Linn.*[20]

Visualization of ligand-protein interactions

1. Quercetin and DPP-4 interaction

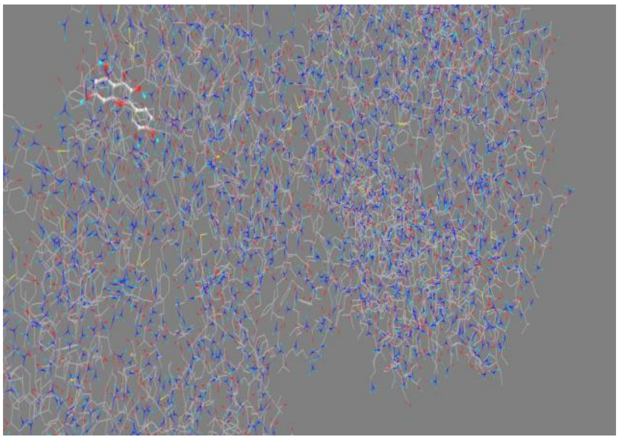


Figure 2: Quercetin+DPP-4 interaction.

The docking analysis of quercetin with DPP-4 showed a binding affinity of -8.9 kcal/mol. The ligand was observed to bind effectively within the active site of the protein, forming stable interactions through hydrogen bonding and hydrophobic forces. These interactions suggest good binding stability and potential inhibitory activity.

2. Quercetin and Alpha-glucosidase interaction

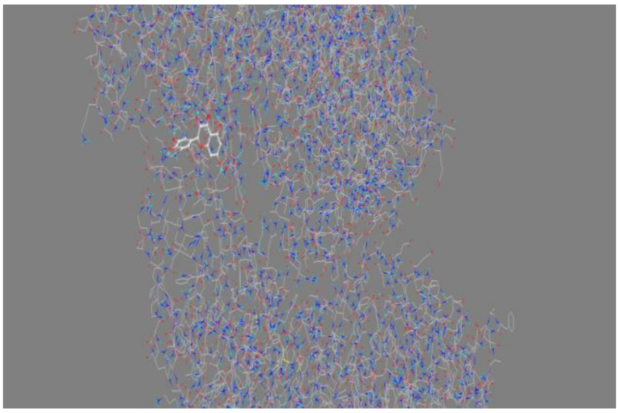


Figure 3: Quercetin+ Alpha glucosidase interaction.

Quercetin exhibited a binding affinity of -8.7 kcal/mol with α-glucosidase. The ligand was positioned within the active site, forming hydrogen bond interactions that contribute to stabilization of the ligand–protein complex, indicating its potential to inhibit carbohydrate digestion.

3. Quercetin and Alpha-amylase interaction

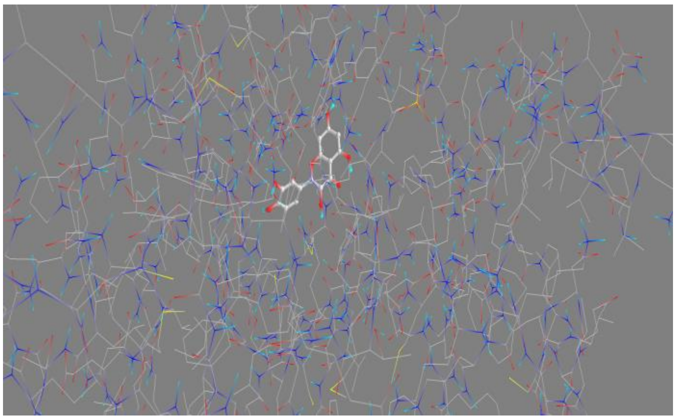


Figure 4: Quercetin + Alpha-amylase interaction.

Quarcetin exhibited good binding affinity with the α-amylase enzyme, showing a docking score of −7.9 kcal/mol. The negative binding energy indicates a stable interaction between the ligand and the target enzyme. These findings suggest that Quercetin may effectively inhibit α-amylase activity and could possess potential antidiabetic properties.

4. Rutin and DPP-4 interaction

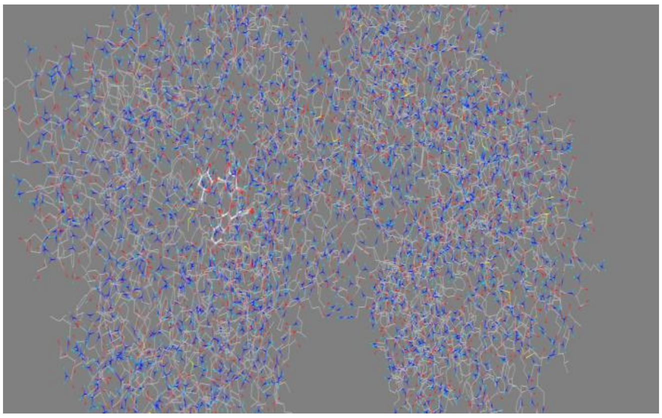


Figure 5: Rutin+DPP-4.

Rutin demonstrated a strong binding affinity of -9.8 kcal/mol with DPP-4. The ligand formed multiple hydrogen bonds due to its polyhydroxylated structure, enhancing binding stability and suggesting potent inhibitory potential.

5. Rutin and Alpha glucosidase interaction

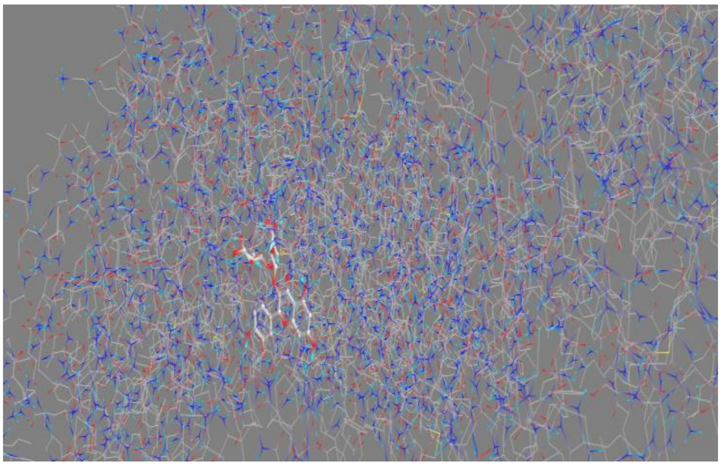


Figure 6: Rutin+ Alpha-glucosidase.

Rutin showed the highest binding affinity of -10.2 kcal/mol with α-glucosidase. The ligand exhibited strong interactions within the active site, including multiple hydrogen bonds, indicating excellent binding stability and significant inhibitory potential.

6. Rutin and Alpha-amylase

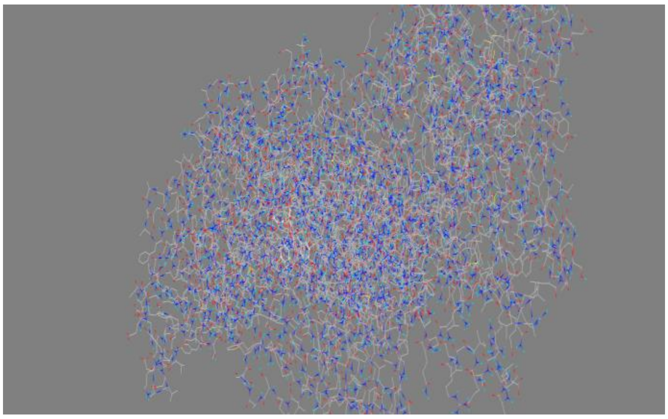


Figure 7: Rutin+ Alpha-amylase interaction.

Rutin demonstrated strong binding affinity with the α-amylase enzyme, with a docking score of −8.4 kcal/mol. The negative binding energy indicates a stable and favorable interaction between the compound and the enzyme active site. These results suggest that Rutin may act as an effective α-amylase inhibitor and could contribute to potential antidiabetic activity.

7. Aegeline and DPP-4 interaction

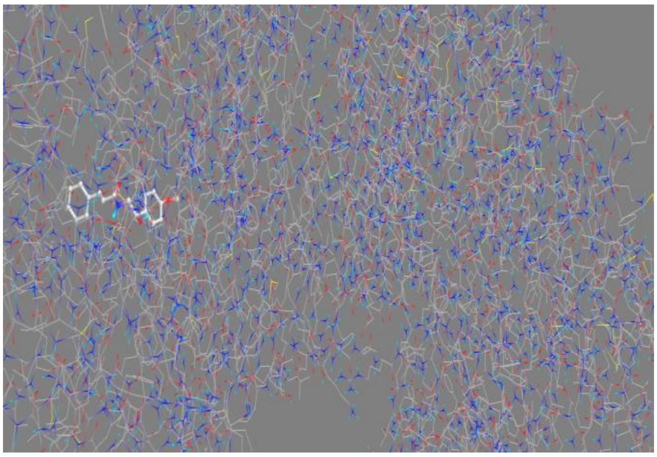


Figure 7: Aegeline+ DPP-4.

Aegeline showed a binding affinity of -7.3 kcal/mol with DPP-4. The ligand formed moderate interactions within the active site, suggesting a comparatively lower but notable inhibitory potential.

8. Aegeline and Alpha-glucosidase

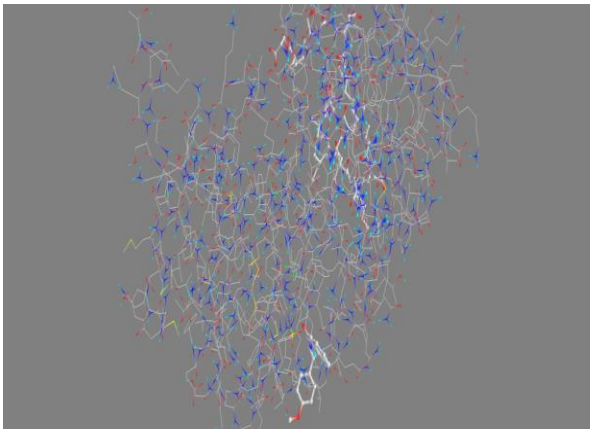


Figure 9: Aegeline+ Alpha-glucosidase interaction.

Aegeline showed good binding affinity with the α-glucosidase enzyme, with a docking score of −8.1 kcal/mol. The negative binding energy indicates a stable interaction between the ligand and the active site of the enzyme. These findings suggest that Aegeline may possess potential antidiabetic activity through inhibition of α-glucosidase, thereby helping to reduce carbohydrate digestion and glucose absorption.

9. Aegeline and Alpha-amylase interaction

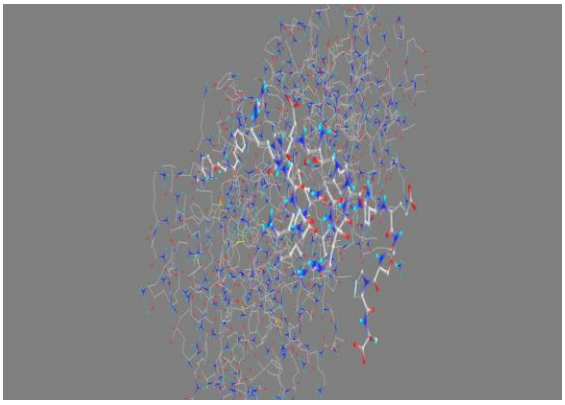


Figure 10: Aegeline+ Alpha-amylase interaction.

Aegeline exhibited good binding affinity with the α-amylase enzyme, showing a docking score of −7.2 kcal/mol. The negative binding energy indicates a stable interaction between the compound and the active site of the enzyme. These results suggest that Aegeline may possess potential antidiabetic activity through inhibition of α-amylase enzyme activity. Binding affinity indicates the strength of interaction between phytoconstituent and target protein.

- More negative docking score = stronger binding
- Strong binding suggests better therapeutic potential

Significant interactions between specific phytoconstituents and diabetes target proteins were shown by the molecular docking investigation. Strong inhibitory activity was suggested by rutin's highest binding affinity against α-glucosidase (-10.2 kcal/mol) and DPP-4 (-9.8 kcal/mol). Additionally, quercetin had a strong affinity for α-glucosidase and DPP-4. The binding affinities of aegeline were moderate. According to the docking results, flavonoids found in *Aegle marmelos Linn.* may efficiently block enzymes that metabolize carbohydrates and enhance glucose management.[21]

Preparation of plant Extract

Collection of plant material

Seasonally, fresh *Aegle marmelos Linn.* leaves were gathered from an uncontaminated source and carefully cleaned to get rid of contaminants. The active ingredients were then preserved by shade-drying them for a few days at room temperature.

Figure 11: *Aegle marmelos* leaves.

Figure 12: Shed dried leaves.

Grinding of plant material

The dried plant material of *Aegle marmelos* was subjected to size reduction by mechanical grinding using a suitable grinder. The grinding process was carried out carefully to obtain a coarse powder while avoiding excessive heat generation, which may degrade the active constituents. Once completely dried, the leaves were coarsely powdered using a grinder and passed through a suitable sieve (e.g., sieve no. 60) to obtain uniform particle size. The powdered material was stored in an airtight container for further experimental use.[22]

Figure 13: Grinding of collected plant material.

Figure 14: Sieving of powder.

3. Extraction of *Aegle marmelos Linn.* powder

Materials Required

Dried bael leaves powder, Solvent: Ethanol, Thimble (cellulose), Round bottom flask (250–500 mL), Heating mantle, Condenser, Weighing balance.

Process

The leaves of *Aegle marmelos Linn*. were extracted using a Soxhlet extractor. About 20–30 g of dried, powdered plant material was placed in a cellulose thimble. Ethanol was added to the round bottom flask of the apparatus. The system was heated, causing the solvent to vaporize, condense in the condenser, and filter through the plant. The extraction continued for several cycles (6–10) until the solvent in the siphon tube was colorless, indicating successful extraction.

Figure 15: Soxhlet Assembly.

Evaporation

After extraction with a Soxhlet extractor, the dissolved phytoconstituents were collected in a round bottom flask. Solvent evaporation was then performed through heating on a water bath at a controlled temperature below the solvent's boiling point, resulting in a semi-solid or dry mass. The process was monitored to avoid overheating, preventing degradation of active constituents. Finally, the concentrated extract was cooled and stored in an airtight container for future use.[23]

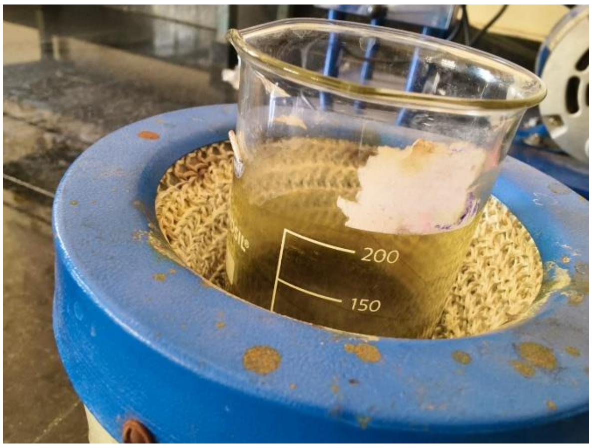


Figure 16: Evaporation on heating mantle.

Figure 17: Extracted Drug.

Preliminary testing

The obtained extract of *Aegle marmelos* was subjected to preliminary phytochemical screening using standard qualitative tests. Small quantities of the extract were treated with specific reagents to detect the presence of different classes of phytochemicals.

- **Test for Tannins**

To 1ml of extract, 2ml of 5% ferric chloride was added. Formation of greenish black color indicated the presence of tannins.

- **Test for Saponins**

To 2ml of extract, 2ml of distilled water was added and shaken in a graduated cylinder for 15minutes lengthwise. Formation of 1cm layer of foam indicated the presence of saponins.[24]

- **Test for Flavonoids**

5ml of dilute ammonia solution was added to a portion of the aqueous filtrate of extract followed by addition of concentrated sulphuric acid. Appearance of yellow colouration indicated the presence of flavonoids.

- **Test for Alkaloids**

To 2ml of extract, 2ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. Presence of green color indicated the presence of alkaloids.

- **Test for Quinones**

To 1ml of extract, 1ml of concentrated sulphuric acid was added. Formation of red color indicated the presence of quinones.[25]

- **Test for Cardiac glycosides**

To 0.5ml of extract, 2ml of glacial acetic acid and few drops of 5% ferric chloride were added. This was under layered with 1ml of concentrated sulphuric acid.

The development of characteristic colour changes or precipitates indicated positive results for particular constituents.[26]

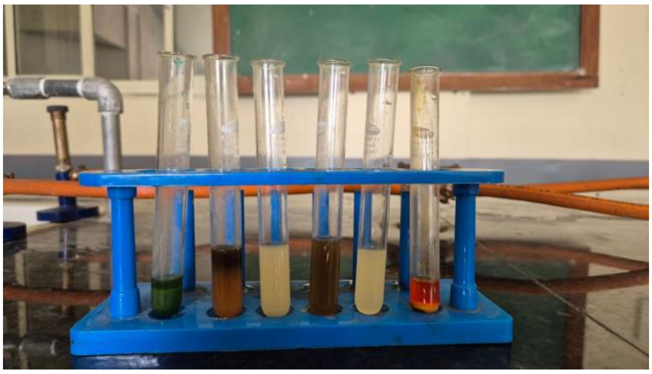


Figure 18: Preliminary Test of Aegle marmelos (leaf powder).

In Vitro Antioxidant Evaluation

Antioxidant Assay

Ferric-Reducing Antioxidant Power (FRAP) Assay Briefly, the reaction mixture included different concentrations of A. marmelos extract (25–250 µg/mL); 0.2M of 2.5 mL of phosphate buffer (pH 6.6); potassium ferricyanide (2.5 mL, 1% w/v). After a 20 min incubation at 50 ◦C, 2.5 mL of trichloroacetic acid (10%) and 0.5 mL of FeCl3 (0.1%) were added. At 700nm, absorbance was recorded in comparison to blank samples (mixture without samples). Positive controls included ascorbic acid. The reducing power of iron from (Fe^{3+}) to Fe^{2+} was evaluated in triplicate.

$$\%Inhibition = \frac{A\ control - A\ sample}{A\ control} \times 100$$

In Vitro Antidiabetic Evaluation

Inhibitory Activity of α-Amylase and α-Glucosidase Different concentrations of A. marmelos extract (25–500 µg/mL) were used to evaluate α-amylase and αglucosidase inhibition potential. Briefly, for the α-amylase assay, 1.0 mL of different concentrations of A. marmelos extract and 1.0 mL α-amylase were mixed by gentle shaking and incubated for 30 min at 37 ◦C; then, 1.0 mL of starch solution was added and the solution was incubated for 1 h at the same condition. Furthermore, 100 µL of supernatant was removed, and the inhibitory activity of A. marmelos extract was measured. For the α-glucosidase assay, 120 µL of different concentrations of A. marmelos extract and 20 µL of α-glucosidase were incubated for 15 min, and the reaction was carried out by adding 20 µL of 5 mM p-nitrophenyl-α-D-glucopyranoside substrate. The reaction was terminated by adding 80 µL potassium phosphate buffer, and absorbance was recorded at 405 nm. Acarbose was employed as a positive control.[27]

$$\%Inhibition = \frac{A\ control - A\ sample}{A\ control} \times 100$$

UV analysis – In vitro Antidiabetic Evaluation.

- Control – Alpha amylase -10mg in 100ml
- 0.1 Mg/ml (100ug/ml)
- Take 1ml in dilute it in 9ml water
- Control Absorbance – 0.0610

Sample Dilution Chart

Concentration (ug/ml)	Stock Solution (ml)	Distilled Water (ml) Final Volume (ml)	Final Volume (ml)
100	1	9	10
200	2	8	10
300	3	7	10
400	4	6	10

Standard sample (Metformin)Uv absorbance readings at 540 nm.

Concentration (ug/ml)	Absorbance	%Inhibition
100	0.0600	1.64%
200	0.0594	2.62%
300	0.0594	8.20%
400	0.0555	9.02%

Test sample UV Absorbance Readings. (Aegle marmelos Extract).

Concentration (ug/ml)	Absorbance	%Inhibition
100	0.0609	0.16%
200	0.0599	1.80%
300	0.0572	6.23%
400	0.0562	7.87%

The in-vitro antidiabetic activity of Aegle marmelos extract was evaluated by α-amylase inhibitory assay and compared with the standard drug Metformin. The UV absorbance readings were recorded at 540 nm.

The standard drug Metformin showed increasing percentage inhibition with increase in concentration. The Aegle marmelos extract also exhibited concentration dependent antidiabetic activity.

The results indicate that the extract possesses significant α-amylase inhibitory activity, although lower than the standard drug Metformin. The increase in inhibition with concentration suggests the presence of phytoconstituents such as flavonoids, tannins and phenolic compounds which may contribute to antidiabetic activity by delaying carbohydrate digestion and glucose absorption.

Overall, the study demonstrates that Aegle marmelos extract has promising antidiabetic potential and could be useful as a natural therapeutic agent for the management of diabetes mellitus.

In vitro Antioxidant Evaluation

- UV Absorbance reading at 700 nm.
- Blank – no standard drug or extract add in dilution
- Blank Absorbance – 0.0615
- Control – 0.7650

Standard sample (Ascorbic acid) UV absorbance reading at 700nm.

Concentration (ug/ml)	Absorbance	%Inhibition
100	0.0819	89.29%
200	0.0704	90.80%
300	0.1039	86.42%
400	0.1374	82.04%

Test Sample (Aegle marmelos extract) UV Absorbance reading at 700nm.

Concentration (ug/ml)	Absorbance	%Inhibition
100	0.5741	24.95%
200	0.5148	32.71%
300	0.6104	20.21%
400	1.6795	-119.54%

The antioxidant activity of Aegle marmelos extract was evaluated using UV absorbance readings at 700 nm and compared with standard Ascorbic acid.

The Aegle marmelos extract exhibited moderate antioxidant activity.

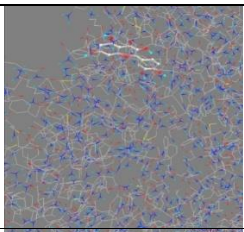
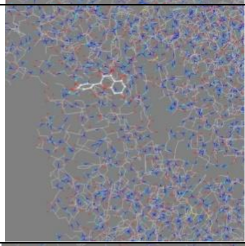
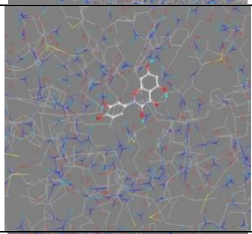
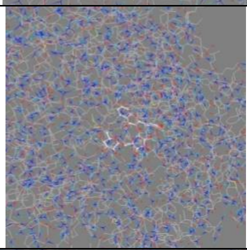
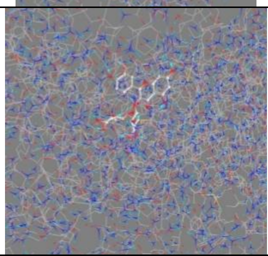
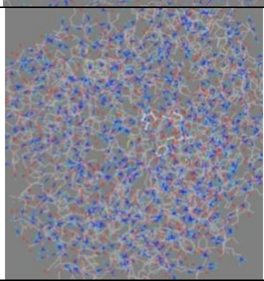
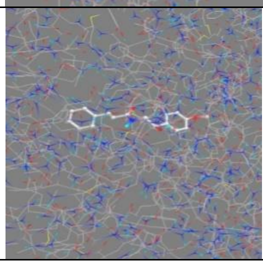
The antioxidant activity of the extract was lower than the standard Ascorbic acid at all concentrations.

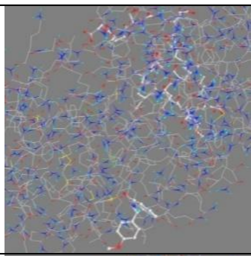
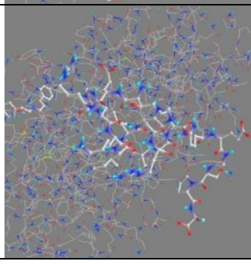
Despite lower activity compared to the standard, the extract demonstrated appreciable antioxidant potential at lower concentrations, which may be attributed to the presence of natural antioxidants such as phenolics, flavonoids and alkaloids in Aegle marmelos.

Thus, the study confirms that Aegle marmelos possesses antioxidant properties, though its activity is comparatively less than standard Ascorbic acid.[27]

RESULTS AND DISCUSSION

In Silico Result

Phytoconstituent+ Protein	Interaction structure	Binding affinity(kcal/mol)
Quercetin + DPP-4		-8.9
Quercetin + Alpha glucosidase		-8.7
Quercetin + Alpha-amylase		-7.9
Rutin+ DPP		-9.8
Rutin+ Alpha-glucosidase		-10.2
Rutin+ Alpha-amylase		-8.4
Aegeline+ DPP-4		-7.3

Aegeline+ Alpha-glucosidase		-8.1
Aegeline+ Alpha-amylase		-7.2

The molecular docking study demonstrated significant interaction between selected phytoconstituents and diabetic target proteins. Rutin exhibited the highest binding affinity against α-glucosidase (-10.2 kcal/mol) and DPP-4 (-9.8 kcal/mol), suggesting strong inhibitory activity. Quercetin also showed considerable affinity toward DPP-4 and α-glucosidase. Aegeline displayed moderate binding affinities. The docking results indicated that flavonoids present in *Aegle marmelos Linn.* may effectively inhibit carbohydrate metabolizing enzymes and improve glucose regulation.

Preliminary Testing Result

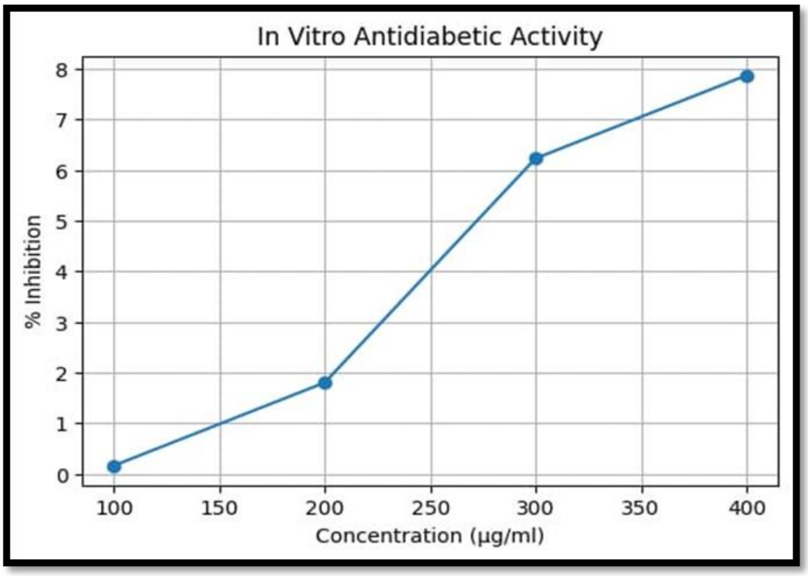
Chemical Constituents	Test	Result
Alkaloids	1.Dragendorff's Test	+
	2.Mayer's Test	+
	3.Wagner's Test	+
	4. Hager's Test	+
Flavonoids	1.Shinoda Test	+
	2.Alkaline Reagent Test	+
	3.Sulphuric Acid Test	+
Saponins	1.Foam Test	+
Glycosides	1.Keller-Killiani Test	+
Tannins	1.Ferric Chloride Test	+
	2.Lead Acetate Test	+
Carbohydrates	1.Molisch's Test	+
	2.Benedict's Test	+

Preliminary test of *Aegle marmelos linn*[25,26,27]

The preliminary phytochemical screening of the extract confirmed the presence of flavonoids, tannins, alkaloids, saponins, quinones, and cardiac glycosides. The phytoconstituents are known to possess antioxidant and antidiabetic activities and may contribute to the pharmacological potential of the plant.

In-vitro Antidiabetic Activity

Concentration (ug\ml)	Absorbance	%Inhibition
100	0.0609	0.16%
200	0.0599	1.80%
300	0.0572	6.23%
400	0.0562	7.87%

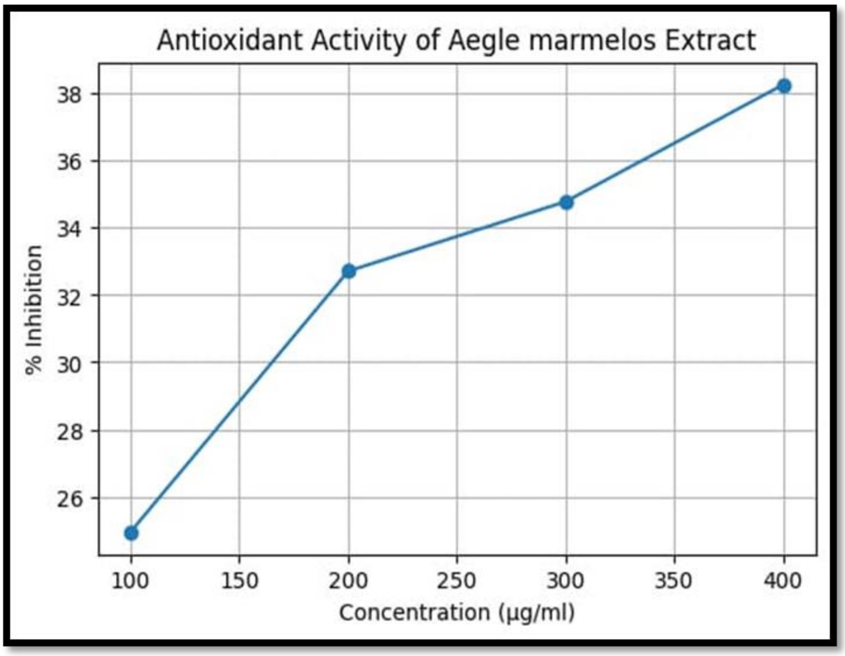


Antidiabetic activity graph.

The Aegle marmelos extract showed concentration-dependent α-amylase inhibitory activity at 540 nm. The activity increased with increase in concentration but was lower than the standard drug Metformin. The presence of flavonoids, tannins and phenolic compounds may contribute to its antidiabetic potential.

In-vitro Antioxidant Activity

Concentration(ug/ml)	Absorbance	%Inhibition
100	0.5741	24.95%
200	0.5148	32.71%
300	0.4989	34.78%
400	0.4723	38.26%



Anti-oxidant activity graph

The Aegle marmelos extract exhibited moderate antioxidant activity at 700 nm when compared with standard Ascorbic acid. Although the activity was lower than the standard, the extract showed appreciable antioxidant potential due to the presence of natural phytoconstituents such as phenolics and flavonoids.

SUMMARY

The present project focused on the in silico and pharmacological evaluation of the antidiabetic and antioxidant potential of secondary metabolites from *Aegle marmelos Linn.* Diabetes mellitus is one of the major health problems worldwide, and oxidative stress is considered an important factor responsible for diabetic complications. Therefore, the study aimed to investigate the role of medicinal plants as safer and economical alternatives for diabetes management.

The selected phytoconstituents such as quercetin, rutin and aegeline were subjected to molecular docking studies against diabetic target proteins including DPP-4, α-glucosidase, α-amylase. The docking studies revealed that rutin and quercetin showed strong binding affinity with target proteins, especially α-glucosidase and DPP-4, suggesting good antidiabetic potential.

The leaves of *Aegle marmelos Linn.* were collected, shade dried, powdered, and extracted using Soxhlet extraction. Preliminary phytochemical screening confirmed the presence of important secondary metabolites.

The in vitro antidiabetic activity was evaluated by α-amylase inhibitory assay using UV spectrophotometry at 540 nm. The extract showed concentration-dependent inhibition, although lower than the standard drug metformin. Antioxidant activity was evaluated using FRAP assay at 700 nm and compared with ascorbic acid. The extract demonstrated moderate antioxidant activity due to the presence of alkaloid and flavonoid compounds.

Overall, the study confirmed that *Aegle marmelos Linn.* possesses significant antidiabetic and antioxidant properties. The correlation between docking results and laboratory findings supports the therapeutic potential of the plant. Further studies involving isolation of active compounds, toxicity studies, and clinical evaluation are required for the development of effective herbal formulations.

CONCLUSION

The present study successfully evaluated the antidiabetic and antioxidant potential of *Aegle marmelos Linn.* using both in silico and in vitro approaches. Molecular docking studies demonstrated that phytoconstituents such as rutin and quercetin exhibited strong binding affinity toward important diabetic target proteins, particularly α-glucosidase and DPP-4, indicating promising inhibitory activity.

Preliminary phytochemical screening confirmed the presence of biologically active compounds including flavonoids, alkaloids, saponins, and glycosides, which are responsible for various pharmacological effects. The in vitro antidiabetic evaluation showed concentration-dependent α-amylase inhibitory activity of the plant extract, while antioxidant studies demonstrated moderate free radical scavenging activity.

The results obtained from laboratory experiments were in agreement with the computational docking studies, suggesting that the phytoconstituents of *Aegle marmelos Linn.* contribute significantly to its therapeutic effects. Therefore, *Aegle marmelos Linn.* can be considered a potential natural source for the development of herbal antidiabetic and antioxidant agents. Further detailed pharmacological, toxicological, and clinical studies are recommended to establish its safety, efficacy, and therapeutic applications.

REFERENCE

1. Arif Malik, Ferdosi MFH, Javed N, Manan A, Rasool M, Qazi MH. Antidiabetic, antihyperlipidemic and antioxidative effects of *Aegle marmelos Linn.* and silymarin on alloxan induced diabetes in mice. Mycopath, 2012; 10(2): 87-90.
2. Shahedur Rahman, Parvin R. Therapeutic potential of *Aegle marmelos Linn.* (L.)–An overview. Asian Pac J Trop Dis., 2014; 4(1): 71-77.
3. Sabu MC, Kuttan R. Antidiabetic activity of *Aegle marmelos Linn.* and its relationship with its antioxidant properties. Indian J Physiol Pharmacol., 2004; 48(1): 81-88.
4. M. Poonkothai, Saravanan M. Antibacterial activity of *Aegle marmelos Linn*. against leaf, bark and fruit extracts. Anc Sci Life, 2008; 17(3): 15-18.
5. Rahul Swarnkar, Singh D, Choudhary A, Anand S, Rathore A, Jediya HK. Pharmacological properties of *Aegle marmelos Linn*.: A review. Int J Curr Microbiol App Sci., 2019; 8(5): 1600-1608.
6. Pushpendra K. Patel, Sahu J, Sahu L, Prajapati NK, Dubey BK. *Aegle marmelos Linn.:* A review on its medicinal properties. Int J Pharm Phytopharmacol Res., 2012; 1(5): 332-341.
7. Amitha Shetty, Fernandes L, Shambhavi D, Mahadev M, Dubey A. Phytochemical and pharmacological profile of *Aegle marmelos Linn.* (L.) Correa: A comprehensive review of therapeutic potential, mechanisms of action, and translational relevance. J Appl Pharm Sci., 2026; 16(2): 6-18.
8. Rishi Kesh Meena, Pareek A, Meena RR. Antimicrobial activity of *Aegle marmelos Linn.* (Rutaceae) plant extracts. Int J MediPharm Res., 2016; 2(1): 1-5.
9. Narender T, Shweta S, Tiwari P, Reddy KP, Khaliq T, Prathipati P, et al. Antihyperglycemic and antidyslipidemic agent from *Aegle marmelos Linn.* Bioorg Med Chem Lett., 2007; 17: 1808-1811.
10. Nevedita, Kumar A. A critical review on the medicinal herb *Aegle marmelos Linn.* (Linn.) Correa. Int J Pharm Sci Res., 2023; 14(5): 2087-2103.
11. Dinesh Kumar Sekar, Kumar G, Karthik L, Bhaskara Rao KV. A review on pharmacological and phytochemical properties of *Aegle marmelos Linn.* (L.) Corr. Serr. (Rutaceae). Asian J Plant Sci Res., 2011; 1(2): 8-17.
12. Patkar Atul N, Desai NV, Ranage AA, Kalekar KS. A review on *Aegle marmelos Linn.:* A potential medicinal tree. Int Res J Pharm., 2012; 3(8): 86-91.

13. Hemant K. Jain, Jaiswal SR. Effect of co-administration of Emblica officinalis and *Aegle marmelos Linn.* extracts for antioxidant and antidiabetic activity. Int J Pharm Pharm Sci., 2019; 11(7).
14. PubChem. Quercetin [Internet]. Bethesda (MD): National Center for Biotechnology Information; 2026 [cited 2026 May 26]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Quercetin
15. PubChem. Rutin [Internet]. Bethesda (MD): National Center for Biotechnology Information; 2026 [cited 2026 May 26]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Rutin.
16. PubChem. Aegeline [Internet]. Bethesda (MD): National Center for Biotechnology Information; [cited 2026 May 26]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Aegeline.
17. RCSB Protein Data Bank. Dipeptidyl peptidase-4, PDB ID: 4A5S [Internet]. Available from: https://www.rcsb.org/structure/4A5S .
18. RCSB Protein Data Bank. Alpha-glucosidase, PDB ID: 3TOP [Internet]. Available from: https://www.rcsb.org/structure/3TOP .
19. RCSB Protein Data Bank. Human pancreatic alpha-amylase, PDB ID: 1HNY [Internet]. Available from: https://www.rcsb.org/structure/1HNY .
20. Rawaba Arif, Ahmad S, Mustafa G, Mahrosh HS, Ali M, Qamar MTU, Dar HR. Molecular docking and simulation studies of antidiabetic agents devised from hypoglycemic polypeptide-P of Momordica charantia. Biomed Res Int., 2021; 2021: 5561129.
21. Bitew M, Desalegn T, Demissie TB, Belayneh A, Endale M, Eswaramoorthy R (2021) Pharmacokinetics and drug-likeness of antidiabetic flavonoids: Molecular docking and DFT study. PLoS ONE 16(12): e0260853.
22. Hemant K. Jain, Jaiswal SR. Effect of co-administration of Emblica officinalis and Aegle marmelos Linn. extracts for antioxidant and antidiabetic activity. Int J Pharm Pharm Sci., 2019; 11(7).
23. C. T. Sulaiman, Shahida V, Balachandran I. Effect of extraction solvent on the phytoconstituents of Aegle marmelos Linn. (L.) Correa. J Nat Remedies, 2015; 15(1).
24. Umadevi Kumba Janarthanan, Varadharajan V, Krishnamurthy V. Physicochemical evaluation, phytochemical screening and chromatographic fingerprint profile of Aegle marmelos Linn. (L.) leaf extracts. World J Pharm Res., 2012; 1(3): 813-837.

25. Nusrat Jahan Bristy, Hasan AHMN, Alam MN, Wahed TB, Roy P, Alam KMK. Characterization of antioxidant and cytotoxic potential of methanolic extracts of different parts of Aegle marmelos Linn. (L.). Int J Pharm Sci Res., 2017; 8(3): 1476-1484.
26. Nirmala Gupta, Agrawal RC, Shrivastava V, Roy A, Prasad P. In vitro antioxidant activity and phytochemical screening of Aegle marmelos Linn. extracts. Res J Pharmacogn Phytochem., 2012; 4(2): 80-83.
27. Ahmad, W.; Amir, M.; Ahmad, A.; Ali, A.; Ali, A.; Wahab, S.; Barkat, H.A.; Ansari, M.A.; Sarafroz, M.; Ahmad, A.; et al. Aegle marmelos Linn. Leaf Extract Phytochemical Analysis, Cytotoxicity, In Vitro Antioxidant and Antidiabetic Activities. Plants, 2021; 10: 2573. https://doi.org/10.3390/ plants10122573