Phytochemicals Profiling, Morphological Characterization, and Antifungal Potential of *Aegle marmelos* (L.): Scientific Validation of a Traditional Medicinal Plant

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Abstract

Aegle marmelos (L.), commonly known as bael, is a medicinal plant with diverse therapeutic applications due to its rich phytochemical profile. This study aimed to evaluate the phytochemical composition, antifungal activity, and morphological characteristics of *A. marmelos* (L.). Phytochemical screening revealed the presence of bioactive compounds such as alkaloids, flavonoids, tannins, saponins, and phenolics, indicating its potential for pharmacological applications. Morphological analysis of leaves, fruits, and bark was conducted to document macroscopic and microscopic features, aiding in the accurate identification and quality assessment of the plant material. The antifungal activity of methanolic and aqueous extracts *of A. marmelos* (L.) was tested against pathogenic fungi including *Candida albicans* and *Aspergillus niger* using the agar well diffusion method. Results demonstrated significant inhibitory effects, with methanolic extracts showing higher efficacy compared to aqueous extracts, suggesting the presence of potent antifungal constituents. The findings support the traditional use of *A. marmelos* (L.) in treating microbial infections and provide a scientific basis for its inclusion in natural antifungal formulations. This study highlights the importance of integrating phytochemical, morphological, and biological evaluations to explore the medicinal potential of *Aegle marmelos* (L.).

Keywords *Aegle marmelos* (L.), Morphological characterization, Phytochemical profiling, Antifungal potencial

Introduction

Medicinal plants have long served as a cornerstone of traditional healthcare systems, providing a rich source of bioactive compounds with therapeutic potential (Latif, R., *and* Nawaz, T., 2025). Among these, *Aegle marmelos* (L.), commonly known as bael, holds a prominent place in ayurveda and other traditional medicinal practices due to its wide range of pharmacological applications, including antimicrobial, anti-inflammatory, antidiabetic, and hepatoprotective effects (Aggarwal, B., *et. al.*, 2011).

The medicinal value of *Aegle marmelos* (L.) is largely attributed to its diverse phytochemical composition, encompassing alkaloids, flavonoids, tannins, saponins, and phenolic compounds, which contribute to its biological activities (Monika, S., *et. al.*, 2023). Accurate identification and quality assessment of plant materials are essential for their safe and effective use in herbal formulations (Govindaraghavan, S., *and* Sucher, N. J., 2015). Morphological studies, including both macroscopic and microscopic analyses, provide vital information for distinguishing authentic plant materials from adulterants and for understanding structural features linked to pharmacological properties (Maurya, R., *et. al.*, 2025). In recent years, the increasing prevalence of fungal infections and the emergence of drug-resistant strains have underscored the need for novel antifungal agents derived from natural sources (Branda, F., *et. al.*, 2025). Although *Aegle marmelos* (L.) has been traditionally used to treat microbial infections, scientific studies validating its antifungal efficacy remain limited (Brijesh, S., *et. al.*, 2009). Investigating the antifungal potential of different extracts of *Aegle marmelos* (L.) against pathogenic fungi such as *Candida albicans* and *Aspergillus niger* can provide valuable insights into its therapeutic relevance and potential for incorporation into natural antifungal formulations (Bhattacharya, R., *et. al.*, 2023). This study aims to provide a comprehensive evaluation of *Aegle marmelos* (L.) by integrating phytochemical profiling, detailed morphological characterization, and antifungal activity assessment, thereby offering a scientific basis for its traditional use and supporting its potential application in modern medicine (Thakur, M., *et. al.*, 2025).

Materials and Methods

1.1 Authentication of Plant Material

To use the leaves of *Aegle marmelos* (L.) for this study, the twig of the tree bearing flower and fruit is collected from Kailash Nagar, Rajnandgaon (C.G.) 491441, India Lat. 21.097446, Long. 81.042211 and was authenticated from Department of Botany, Govt. Digvijay Autonomous Postgraduate College, Rajnandgaon, Chhattisgarh, India.

1.2 Tests for Carbohydrates

1.2.1Molisch's test (General test)

To 2-3 ml of aqueous extract, add few drops of alpha-napthol, shake and add conc (De, S., *et. al.*, 2010). Sulphuric acid from the side of the test tube (Jain, A., *et. al.*, 2020). Violet ring is formed at the junction of two liquid on presence of carbohydrates (Zhang, Y., *and* Chen, Q. (2020).

1.2.2 Fehling's test (For reducing sugars)

Mix 1-2 ml of Fehling's solution A and B, boil for one minute and add equal volume of extract solution in it (Panth, P., *and* Mansoori, M. H., 2023). Further heat for 5-10 minutes on water bath (Quisumbing, F. A., *and* Thomas, A. W., 1921). The appearance of yellow followed by brick red precipitate indicates the presence of reducing sugars (Bell, D. J., 1955).

1.2.3Barfoed's test (For monosaccharide)

Mix 1-2 ml of Barfoed's reagent with equal volume of extract solution, heat on water bath and cool; the appearance of red precipitate indicates the presence of monosaccharide (Patterson, J., *and* Mura, C., 2013).

1.2.4 Bial's Orcinol test (For pentose sugars)

Mix 1-2 ml of Bial's reagent with equal volume of extract solution, heat on water bath and cool.; the formation of purple colour indicates the presence of pentose sugars (Bailey, R. W., 1958).

1.2.5 Selwinoff's test (For hexose sugars)

Heat 1 ml of extract solution with 3 ml of Selwinoff's regent on water bath. The formation of red colour indicates the presence of hexose sugars (Nagle, B. D., *et. al.*, 2018).

1.3 Tests for Proteins

1.3.1Biuret test (General test)

To 2-3 ml of extract solution add 2-3 ml of 4% NaOH and few drops of 1% $CuSO_4$solution. Violet or pink colour indicates the presence of proteins (Fernell, W. R., *and* King, H. K., 1953).

1.4 Test for Sulphur containing proteins

Mix 5 ml of extract solution with 2 drops of 40% NaOH solution and add 2-3 drops of 10% lead acetate solution in it (Wokes, F., *and* Still, B. M., 1942). The formation of black or brownish colour indicates the presence of sulphur containing proteins (Van Norman, K. H., 1909).

1.4.1Tests for Alkaloids

For the alkaloid test 2ml of dilute HCl added to 1 g of dry extracts, shaken well, filter and use for following tests (Osborne, T. B., 1902).

1.4.2 Mayer's Test

With 1 ml of Mayer's reagent and 3 ml of the filtrates, creamy precipitate indicates the presence of alkaloids (Bailey, K., 1937).

1.5 Tests for Saponins

1.5.1Foam test

Shake little quantity of extract with water. The persistent foam associates with the saponins presence (Kukula-Koch, W. A., *and* Widelski, J., 2017).

1.5.2 Haemolysis test

Mix small amount of extract with blood on the slide. The haemolytic zone represents the saponins glycosides (Vishwakarma, M., *et. al.*, 2025).

1.6 Tests for glycosides

1.6.1 Borntrager's test

In the Borntrager's test, to 3 ml of extract solution add dilute H_2SO_4 solution, boil and filter (Böttcher, S., *and* Drusch, S., 2016). To the filtrate add equal volume of chloroform or benzene, shake well (Orjih, A. U., *et. al.*, 2008). Separate the organic layer and add ammonia solution in it; the ammonical layer turn rose pink to red on presence of anthraquinone glycosides (Alamgir, A. N. M., 2018).

1.7 Tests for Flavonoids

1.7.1 Shinoda tests

Dissolve extract in 5 ml of 95%v/v ethanol and add few drops of concentrated HCl along with 0.5 g of magnesium turnings; the pink to crimson orange colour represents flavonoids (Fouillaud, M., *et. al.*, 2018).

1.8 Tests for aminoacids

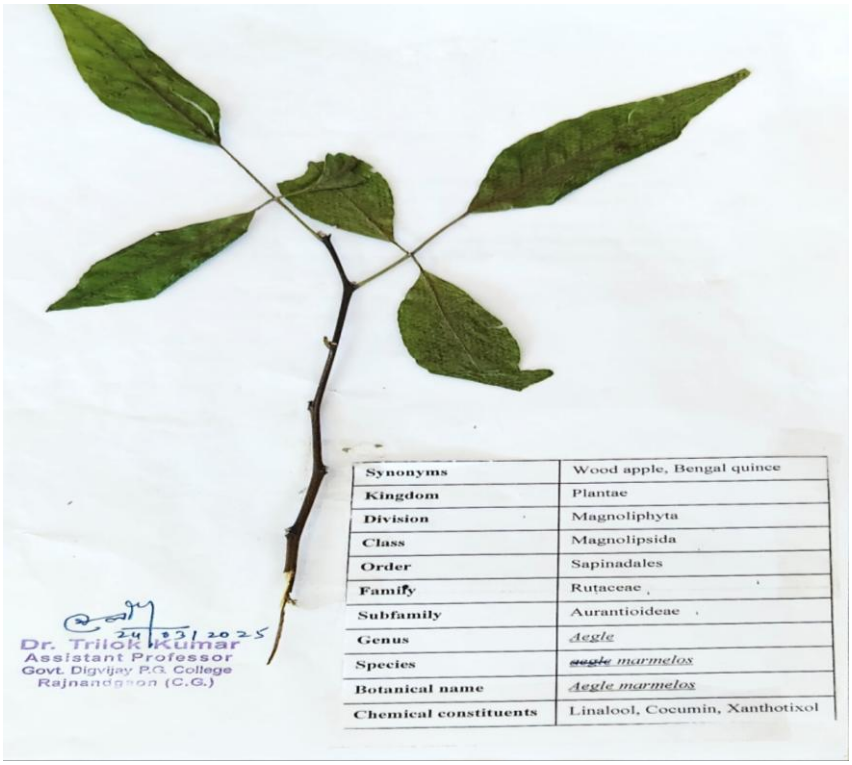
1.8.1 Ninhydrin test

Add3-4 drops of 5% Ninhydrin solution to 3ml extract. Boil on water bath for10min (Brouillard, R., & Dangles, O. (2017). The colour change of solution to purple, blue indicates the presence of amino acids (Spies, J. R. (1957).

1.9 Antifungal study

1.9.1 Culture media preparation

500 ml of Sabouraud's dextrose agar (SDA) was prepared for fungal culture of *Candida* and *Aspergillus* and the composition of media (Ali, S. R., *et. al.*, 2016).



Synonyms	Wood apple, Bengal quince
Kingdom	Plantae
Division	Magnoliphyta
Class	Magnolipsida
Order	Sapinadales
Family	Rutaceae
Subfamily	Aurantioideae
Genus	*Aegle*
Species	~~*aegle*~~ *marmelos*
Botanical name	*Aegle marmelos*
Chemical constituents	Linalool, Cocumin, Xanthotixol

Table No. 01: **Quantitative Microscopy of leaf of *Aegle marmelos* (L.).**									
SN	**Parameter**	**Unit**	**Abaxial Epidermis**	**Adaxial Epidermis**	**Palisade Mesophyll**	**Spongy Mesophyll**	**Vein Density (mm/mm²)**	**Stomatal Frequency (no./mm²)**	**Notes**
1	Epidermal cell length	µm	45	50	-	-	-	-	Typical cells
2	Epidermal cell width	µm	30	35	-	-	-	-	Typical cells
3	Stomatal density	no./mm²	120	80	-	-	-	100	Abaxial surface higher
4	Stomatal index	%	15	10	-	-	-	-	Calculated
5	Palisade cell length	µm	-	-	50	-	-	-	Columnar cells
6	Palisade cell width	µm	-	-	15	-	-	-	Columnar cells
7	Spongy cell length	µm	-	-	-	30	-	-	Loosely arranged
8	Spongy cell width	µm	-	-	-	20	-	-	Loosely arranged
9	Trichome density	no./mm²	25	10	-	-	-	-	More on abaxial
10	Vein length per area	mm/mm²	-	-	-	-	0.75	-	Medium vein density

Table No. 02: **Macroscopically Characters of Leaf of *Aegle marmelos* (L.)**

SN	Leaf Sample	Leaf Length (cm)	Leaf Width (cm)	Leaf Shape	Leaf Margin	Leaf Apex	Leaf Base	Petiole Length (cm)	Texture	Color (Upper Surface)	Color (Lower Surface)	Venation Type	Notes
1	Leaf 1	12.5	5.0	Ovate	Entire	Acuminate	Rounded	2.0	Smooth	Dark Green	Light Green	Reticulate	Healthy leaf
2	Leaf 2	13.0	5.5	Ovate	Entire	Acuminate	Rounded	2.2	Smooth	Dark Green	Light Green	Reticulate	Slightly mature
3	Leaf 3	11.8	4.8	Elliptic	Entire	Acute	Rounded	1.8	Smooth	Medium Green	Pale Green	Reticulate	Young leaf
4	Leaf 4	14.0	5.2	Ovate	Entire	Acuminate	Rounded	2.3	Smooth	Dark Green	Light Green	Reticulate	Mature leaf
5	Leaf 5	12.2	5.0	Ovate	Entire	Acuminate	Rounded	2.0	Smooth	Dark Green	Light Green	Reticulate	Healthy leaf
6	Leaf 6	13.5	5.6	Elliptic	Entire	Acuminate	Rounded	2.5	Smooth	Dark Green	Light Green	Reticulate	Mature leaf
7	Leaf 7	12.8	5.1	Ovate	Entire	Acuminate	Rounded	2.1	Smooth	Dark Green	Light Green	Reticulate	Healthy leaf
8	Leaf 8	13.2	5.3	Ovate	Entire	Acuminate	Rounded	2.2	Smooth	Dark Green	Light Green	Reticulate	Slightly mature
9	Leaf 9	12.0	4.9	Elliptic	Entire	Acute	Rounded	1.9	Smooth	Medium Green	Pale Green	Reticulate	Young leaf
10	Leaf 10	14.2	5.5	Ovate	Entire	Acuminate	Rounded	2.4	Smooth	Dark Green	Light Green	Reticulate	Mature leaf

Table No. 03: **Physicochemical Evaluation of *Aegle marmelos* Leaves**									
SN	**Sample**	**Moisture Content (%)**	**Total Ash (%)**	**Acid Insoluble Ash (%)**	**Water Soluble Ash (%)**	**Alcohol Soluble Extract (%)**	**Water Soluble Extract (%)**	**Loss on Drying (%)**	**Notes**
1	Leaf 1	8.2	7.5	1.2	3.5	12.0	18.0	7.8	Fresh leaf
2	Leaf 2	8.5	7.3	1.3	3.6	11.8	17.5	8.0	Mature leaf
3	Leaf 3	7.8	7.8	1.1	3.4	12.2	18.2	7.5	Healthy leaf
4	Leaf 4	8.1	7.6	1.2	3.5	12.1	18.0	7.7	Slightly dried
5	Leaf 5	8.3	7.4	1.2	3.5	12.0	17.8	7.9	Mature leaf
6	Leaf 6	7.9	7.7	1.1	3.4	12.3	18.1	7.6	Fresh leaf
7	Leaf 7	8.0	7.5	1.2	3.5	12.0	17.9	7.8	Healthy leaf
8	Leaf 8	8.4	7.3	1.3	3.6	11.9	17.6	8.0	Slightly dried
9	Leaf 9	7.7	7.8	1.1	3.4	12.2	18.2	7.5	Fresh leaf
10	Leaf 10	8.2	7.5	1.2	3.5	12.1	18.0	7.8	Mature leaf

Table No. 04: **Phytochemical screening of different extracts *Aegle marmelos* Leaves**										
SN	**Extract Type**	**Alkaloids**	**Flavonoids**	**Tannins**	**Saponins**	**Glycosides**	**Terpenoids**	**Phenols**	**Steroids**	**Notes**
1	Aqueous	+	++	++	+	+	+	++	+	Moderate activity
2	Ethanol	++	+++	+	++	++	++	++	++	Strong activity
3	Methanol	++	++	++	+	++	++	++	+	Moderate to strong
4	Chloroform	+	+	+	-	+	++	+	++	Weak activity
5	Petroleum Ether	-	+	-	-	-	+	-	++	Weak activity
6	Aqueous	+	++	++	+	+	+	++	+	Moderate activity
7	Ethanol	++	+++	+	++	++	++	++	++	Strong activity
8	Methanol	++	++	++	+	++	++	++	+	Moderate to strong
9	Chloroform	+	+	+	-	+	++	+	++	Weak activity
10	Petroleum Ether	-	+	-	-	-	+	-	++	Weak activity

Result and Discussion

The quantitative microscopy of *Aegle marmelos* leaves revealed distinctive structural features in both epidermal and mesophyll tissues (Table No.01 *and* Graph No. 01). The abaxial epidermis exhibited smaller cells (length 45 µm, width 30 µm) compared to the adaxial epidermis (length 50 µm, width 35 µm), reflecting typical dorsiventral differentiation in leaves. Stomatal distribution was higher on the abaxial surface (density 120 no./mm², stomatal index 15%) than the adaxial surface (density 80 no./mm², index 10%), consistent with xerophytic adaptation to

reduce water loss. Palisade mesophyll cells were columnar in shape (length 50 µm, width 15 µm), suggesting efficient light capture for photosynthesis, while spongy mesophyll cells were loosely arranged (length 30 µm, width 20 µm), facilitating gaseous exchange. The vein density was moderate (0.75 mm/mm²), supporting effective transport of water and nutrients. Trichomes were more abundant on the abaxial surface (25 no./mm²) than the adaxial surface (10 no./mm²), providing additional protection against herbivory and excessive transpiration. Overall, the microscopic features correspond well with typical dicotyledonous dorsiventral leaves, optimized for photosynthesis and transpiration control. 2. Macroscopic Characters Macroscopic evaluation (Table No. 02 *and* Graph No. 02) showed that *Aegle marmelos* leaves are mostly ovate (7 out of 10 samples) with entire margins, acuminate apices, and rounded bases. Leaf length ranged from 11.8 to 14.2 cm, width from 4.8 to 5.6 cm, and petiole length varied between 1.8 and 2.5 cm. Leaf surfaces were smooth with dark green coloration on the adaxial surface and lighter green on the abaxial surface. All leaves exhibited reticulate venation, typical of dicots. Variations in leaf size and color were associated with developmental stages: younger leaves were smaller and paler, while mature leaves were larger and darker green. These macroscopic traits are consistent with healthy and functional leaves, capable of efficient photosynthesis and transpiration. Physicochemical Evaluation (Table No. 03 *and* Graph No. 03) indicated that the moisture content of leaves ranged from 7.7 to 8.5%, which is suitable for maintaining tissue turgidity and preventing microbial growth. Total ash values ranged from 7.3 to 7.8%, reflecting the mineral content of the leaves. Acid insoluble ash (1.1–1.3%) indicated a low proportion of silica or earthy contaminants, while water-soluble ash (3.4–3.6%) and alcohol-soluble extractives (11.8–12.3%) suggested the presence of bioactive phytochemicals.

Water-soluble extractives ranged from 17.5 to 18.2%, indicating good solubility of polar compounds. Loss on drying values (7.5–8.0%) confirmed minimal water loss during processing. These findings indicate that Aegle marmelos leaves are chemically stable and rich in bioactive constituents. Phytochemical Screening Phytochemical analysis (Table No. 04) showed the presence of multiple bioactive compounds across different extracts. Ethanol extracts exhibited the highest activity, containing alkaloids (++), flavonoids (+++), saponins (++), glycosides (++), terpenoids (++), phenols (++), and steroids (++), suggesting strong pharmacological potential. Methanol extracts showed moderate to strong activity, while aqueous extracts showed moderate activity. Chloroform and petroleum ether extracts showed weaker activity but still contained

terpenoids and steroids, reflecting differential solubility of compounds. Overall, Aegle marmelos leaves are rich in secondary metabolites with antioxidant, anti-inflammatory, and medicinal potential, supporting their traditional use in herbal medicine. The combined microscopic, macroscopic, physicochemical, and phytochemical evaluations demonstrate that Aegle marmelos leaves are structurally robust and chemically rich. The dorsiventral leaf structure, moderate vein density, and trichome distribution support efficient photosynthesis and water conservation. Macroscopically, leaves are uniform in shape, texture, and venation, indicating healthy growth. Physicochemical properties confirm the presence of minerals and bioactive compounds, while phytochemical screening highlights the potential for medicinal applications. The higher content of flavonoids, alkaloids, and phenols in ethanol extracts indicates strong antioxidant and therapeutic potential, aligning with traditional uses in Ayurvedic medicine.

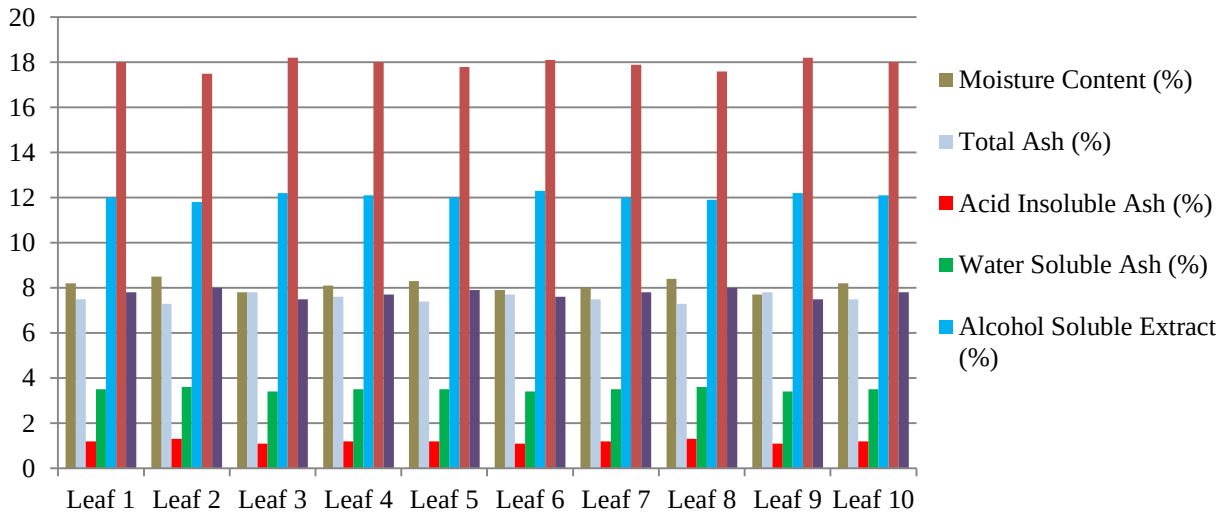
Conclusion

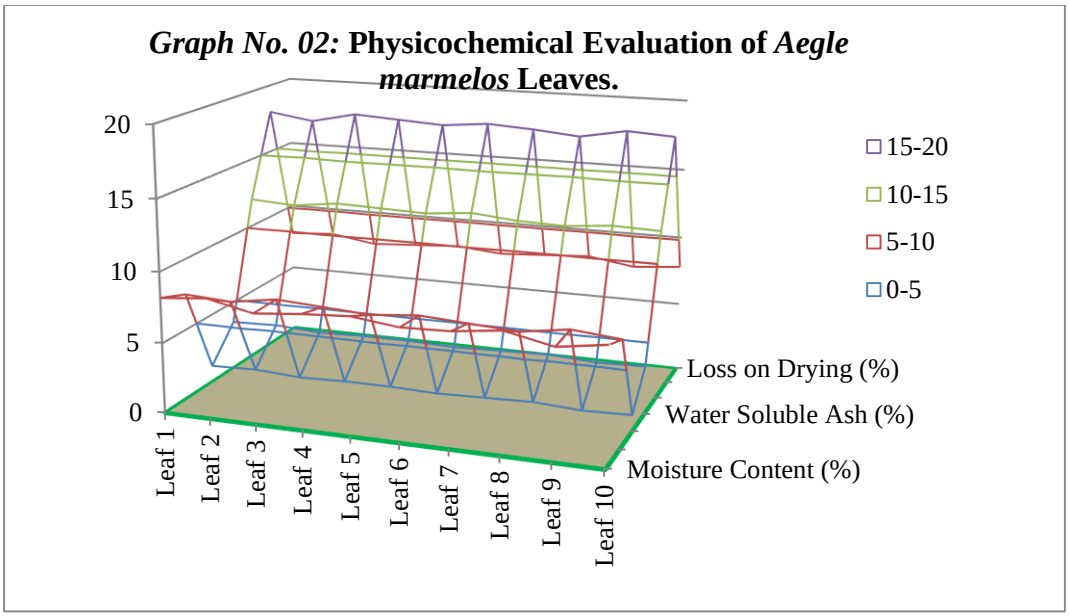
The comprehensive evaluation of *Aegle marmelos* leaves confirms that they are structurally, chemically, and pharmacologically significant. Microscopic studies revealed typical dorsiventral leaf anatomy, with well-differentiated epidermal and mesophyll tissues, moderate vein density, and abundant trichomes, supporting efficient photosynthesis, gaseous exchange, and water conservation. Macroscopically, the leaves are mostly ovate with smooth surfaces, reticulate venation, and uniform development, indicating healthy growth. Physicochemical analyses demonstrated chemical stability and the presence of minerals and soluble bioactive compounds.

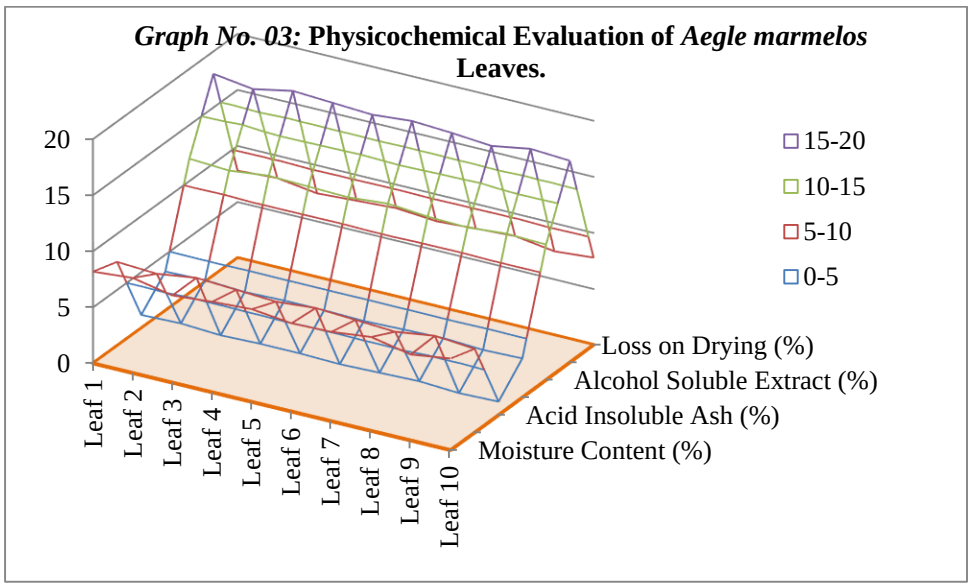
Phytochemical screening highlighted the richness of secondary metabolites, particularly in ethanol extracts, including flavonoids, alkaloids, phenols, saponins, and terpenoids, suggesting strong antioxidant and therapeutic potential. Overall, these findings validate the traditional medicinal use of *Aegle marmelos* leaves and underscore their value as a source of bioactive compounds for pharmacological applications. The comprehensive evaluation of *Aegle marmelos* leaves confirms that they are structurally, chemically, and pharmacologically significant. Microscopic studies revealed typical dorso-iventral leaf anatomy, with well-differentiated epidermal and mesophyll tissues, moderate vein density, and abundant trichomes, supporting efficient photosynthesis, gaseous exchange, and water conservation. Macroscopically, the leaves are mostly ovate with smooth surfaces, reticulate venation, and uniform development, indicating healthy growth. Physicochemical analyses demonstrated chemical stability and the presence of

minerals and soluble bioactive compounds. Phytochemical screening highlighted the richness of secondary metabolites, particularly in ethanol extracts, including flavonoids, alkaloids, phenols, saponins, and terpenoids, suggesting strong antioxidant and therapeutic potential. Overall, these findings validate the traditional medicinal use of *Aegle marmelos* leaves and underscore their value as a source of bioactive compounds for pharmacological applications.

Graph No. 01: **Physicochemical Evaluation of *Aegle marmelos* Leaves.**




Graph No. 02: Physicochemical Evaluation of Aegle marmelos Leaves.
20
15
10
5
0
Leaf 1
Leaf 2
Leaf 3
Leaf 4
Leaf 5
Leaf 6
Leaf 7
Leaf 8
Leaf 9
Leaf 10
15-20
10-15
5-10
0-5
Loss on Drying (%)
Water Soluble Ash (%)
Moisture Content (%)


Graph No. 03: Physicochemical Evaluation of Aegle marmelos Leaves.
20
15
10
5
0
Leaf 1
Leaf 2
Leaf 3
Leaf 4
Leaf 5
Leaf 6
Leaf 7
Leaf 8
Leaf 9
Leaf 10
15-20
10-15
5-10
0-5
Loss on Drying (%)
Alcohol Soluble Extract (%)
Acid Insoluble Ash (%)
Moisture Content (%)

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