

Comparative Investigation of *Aegle Marmelos* Linn. Seeds and Barks for Anti- Cancer Activity against Cell Line: *In-Vitro* Evidence

Ms. Mansi S. Kajale¹, Ms. Dhanashree A. Patil¹, Ms. Sakshi S. Rathod¹, Ms. Snehal S. Kadam¹, Mr. Atharva S. Nimse¹, Mr. Rahul N. Patil^{2*}, Dr. M.R.N Shaikh³

¹Research Scholar, Department of Pharmacology, MET's Institute of D.Pharmacy, BKC, Adgaon, Nashik, Maharashtra, INDIA.

²Assistant Professor, Department of Pharmacology, MET's Institute of D.Pharmacy, BKC, Adgaon, Nashik, Maharashtra, INDIA.

³Principal and Professor, Department of Pharmacognosy, MET's Institute of D.Pharmacy, BKC, Adgaon, Nashik, Maharashtra, INDIA.

Correspondence

Mr. Rahul N. Patil

Assistant Professor, Department of Pharmacology, MET Institute of D.Pharmacy, BKC, Adgaon, Nashik, Maharashtra-422003, INDIA, E-mail: rahulp_iodp@bkc.met.edu.

Received Date: 14 June 2025

Revised Date: 21 July 2025

Accepted Date: 03 August 2025

Publication Date: 12 August 2025

Copyright

© 2025 CME Online Library. This is an open- access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

ABSTRACT

This study explores the comparative anti-cancer potential of *Aegle marmelos* Linn. (Bael) seed and bark extracts against MCF-7 human breast cancer cell lines using *in-vitro* assays (MTT and SRB). Extracts were prepared using ethanol and water and evaluated for phytochemical constituents, physical parameters, and cytotoxic efficacy. Phytochemical analysis confirmed the presence of alkaloids, tannins, saponins, and flavonoids. The ethanolic bark extract demonstrated a significant dose-dependent cytotoxic effect, achieving an IC₅₀ of 82.17 µg/ml in the MTT assay and 44.03 µg/ml in the SRB assay. In contrast, the aqueous seed extract showed comparatively lower activity. These findings suggest that the ethanolic bark extract of *Aegle marmelos* exhibits notable anti-cancer activity and has the potential to be developed into a plant-based therapeutic agent for breast cancer treatment.

KEYWORDS

Aegle marmelos Linn, MCF-7 cell line, Anti-cancer activity.

INTRODUCTION

Cancer is a severe health issue and the second leading cause of death worldwide. It occurs when abnormal cells, either tumor or cancer cells, infiltrate normal tissues and multiply uncontrollably. These cells often detach from their original invasion site and spread through the blood and lymphatic systems, causing metastasis or metastatic spread. Over 100 types of cancer exist, with common ones including lung, stomach, liver, colon, and breast cancer [1].

Breast cancer originates in the cells of the breast tissue. The inner lining of milk ducts or the lobules that supply them with milk is where breast cancer generally begins. A cancerous tumor can metastasize, meaning it can spread to other parts of the body. Lobular carcinoma is a type of breast cancer that begins in the lobules and progresses to the ducts, whereas ductal carcinoma begins in the ducts. Breast cancer is a prevalent and deadly disease. In some regions, like among Nigerian women, it's the most common cancer and leading cause of cancer deaths. The cancer originates in the breast and can spread to lymph nodes, lungs, liver, bones, and brain. Treatment options typically involve surgery, chemotherapy, radiation therapy, or a combination of these approaches. Despite these therapeutic options,

the death rate for breast cancer remains relatively high. Because of these flaws, there is a need for improved therapeutic choices that would raise the likelihood of breast cancer patients surviving with minimum or no therapy adverse effects. Because roughly 5% of human breast cancer is attributable to the inheritance of a breast cancer susceptibility gene, early diagnosis and prevention of breast cancer has been suggested as a superior approach of managing breast cancer [3,4].

National Cancer Institute has evaluated about 35,000 plant species for Anti-Cancer properties. Around 3,000 species have been found to have Anti-cancer activity that can be replicated. *Aegle marmelos* Linn. is a medicinal herb mentioned in ayurveda and it contain a variety of phytochemicals constituent that have been linked to a variety of pharmacological effects like Anti-diabetic activity, Anti-inflammatory activity, Anti-malarial activity, Antimicrobial activity, Antioxidant activity, Anti-acne activity, Hepatoprotective effect, Analgesic activity [2]. *Aegle marmelos* Linn. contain flavanoids and phenolic compounds named marmelosin, marmelin and rutin which are known for their anti-cancer properties. Keeping in this mind research was conducted on title "Comparative Investigation of *Aegle marmelos* Linn. Seeds and Bark for Anti-cancer Activity against Cell line: *In-vitro* Evidence".



MATERIAL AND METHODS

Plant material Collection and Authentication

Plant material Collection and Authentication of *Aegle marmelos* Linn. belonging to family Rutaceae, was collected from local market of Nashik region and the plant was confirmed by Google lens and by HOD of our pharmacognosy department.

Drying and pulverizing of plant material

The plant material (Seed) was dried completely to remove moisture. Powder was made by grinding it finely using a grinder or blender for better extraction. The plant powder was weighed exactly 20-25 g. Plant material (Bark) was dried thoroughly in shade or at 40-45°C in a hot air oven. Powder was made by grinding it using a grinder or mortar-pestle. known amount (e.g., 100 g) of plant powder was weighed.

EXTRACTION OF PLANT MATERIAL

Extraction of seeds

Preparation of extract of plant material (Seed) Powdered plant material was placed into a cellulose thimble or wrapped it in filter paper. The thimble was inserted into the Soxhlet chamber. 250-300 mL of ethanol was poured into the round-bottom flask. The Soxhlet apparatus was set up with the round-bottom flask connected to the condenser. cooling water circulation started in the condenser. The round-bottom flask was heated using a heating mantle or water bath at 50-70°C. The ethanol vaporized, then condensed and dripped into the Soxhlet chamber. The solvent percolated through the plant material, extracting bioactive compounds. When the Soxhlet chamber filled, the solvent siphons back into the round-bottom flask. Extraction was continued for 4-8 hours (or until the extract color fades). Extract was filtered after the extraction was completed. Ethanol was removed using a rotary evaporator (at 40-50°C under reduced pressure). A thick, concentrated extract remained after ethanol evaporation. The extract was dried (in a desiccator or oven at low temp) and Stored in airtight container, preferably at low temperature to prevent degradation [5,6].

Extraction of Bark

Preparation of extract of plant material (Bark) Powdered plant material was taken in a clean, dry conical flask or percolator. Added sufficient solvent (e.g., 500 ml of ethanol for 100 g of powder) to fully immerse the powder. Flask was covered with a stopper or wrapped with aluminum foil. Let it stand for 24-48 hours at room temperature with occasional shaking or stirring to enhance extraction. After maceration, filtered the solution using muslin cloth or Whatman filter paper to collect the extract. Percolation process was repeated with fresh solvent 2-3 times to ensure complete extraction. The extract was dried completely and stored in airtight container [5,6].

Physical Characterization and Phytochemical Study

Beside authentication, crude drugs undergo testing for quality and purity, which involves evaluating parameters such as total ash value, acid insoluble ash value, water soluble ash, sulphated ash, loss on drying, alcohol soluble extractive value, water soluble extractive value, petroleum ether extractive value, foaming index. The extract so collected was evaluated for Carbohydrates, Proteins Fats and Oils, Glycosides, Flavonoids, Alkaloids, Terpenoids, Steroids, Saponins, Tannins and Phenolic Compounds.

PHARMACOLOGICAL STUDY

In-vitro evaluation of Anti-cancer activity

Determination of IC50 by using MTT assay (In-vitro assessment) (23,24).

Cell line – MCF-7 (Human Breast Cancer Cell line)

Media

MEM Medium with high glucose (CatNo-11965-092), FBS (Gibco, Invitrogen) CatNo-10270106, Antibiotic Antimycotic100X solution (Thermo fisher Scientific) CatNo-15240062

Experimental procedure

MCF-7 (Human Mammary gland Breast Adenocarcinoma Cell line) was procured from National center for cell sciences (NCCS), Pune maintained in MEM Medium supplemented with 10 % fetal bovine serum. Cells were cultured at a density of 1×10^4 cells/mL in medium for 24 hours at 37°C with 5% CO₂. Cells were seeded at a concentration (70µl) 104 cells/well in 100µl culture medium and 100µl Samples (10-100µg/ml) into microplates respectively (tissue culture grade, and 96 wells). Control wells were incubated with DMSO (0.2% in PBS) and cell line. All samples were incubated in triplicate. Control groups were maintained to assess baseline cell survival and determine the percentage of viable cells after culturing. Cell cultures were incubated for 24 h at 37°C and 5% CO₂ in CO₂ incubator (Thermo scientificBB150) After incubation, the medium was completely removed and Added 20µl of MTT reagent (5mg/min PBS). After addition of MTT, cells incubated for 4hrs at 37oC in CO₂ incubator. The wells were examined microscopically for formazan crystal formation. Only viable cells reduced the yellow MTT to dark-colored formazan crystals. After removing the medium completely. Added 200 µl of DMSO (kept for 10 min) and incubate at 370°C (wrapped with aluminum foil). Samples were analysed in triplicate by measuring absorbance at 570 nm using an ELISA microplate reader (Benesphera E 21).

Determination of IC50 by using SRB assay

Cell line: MCF-7 (Human Breast cancer cell line)

Media

MEM Medium with high glucose (CatNo-11965-092), FBS (Gibco, Invitrogen) CatNo-10270106, Antibiotic- Antimycotic100Xsolution (Thermo fisher Scientific) CatNo-15240062.

Experimental procedure

MCF-7 (Human Mammary gland Breast Adenocarcinoma Cell line) was procured from National center for cell sciences (NCCS), Pune maintained in MEM Medium supplemented with 10 % fetal bovine serum. Cells were incubated at a concentration of 1×10^4 cells/ml in culture medium for 24 h at 37°C and 5 % CO₂. Cells were seeded at a concentration (70 µl) 104 cells/well in 100 µl culture medium and 100 µl Samples (10-100 µg/ml) into microplates respectively (tissue culture grade, and 96 wells). Control wells were incubated with DMSO (0.2% in PBS) and cell line. All samples were incubated in triplicate. Controls were maintained to determine the control cell survival and the percentage of live cells after culture. Cell cultures were incubated for 24 h at 37°C and 5% CO₂ in CO₂ incubator (Thermo scientific BB150). After incubation, the medium was completely removed and Add 50 µL of 0.4% SRB solution (in 1 % acetic acid) to each well. Incubate at room temperature for 30 minutes. Wash the wells 5 times with 1% acetic acid to remove unbound dye. Air dry the plate completely. Add 100 µL of 10 mM Tris base (pH 10.5) to each well. Shake the plate for 5-10 minutes to dissolve bound dye). Measure absorbance at 560 nm using a microplate reader [7,8].

Statistical Analysis

All the data were expressed as SEM. Statistical comparison was performed on graph pad prism, version 8.03 by two way ANOVA followed by Dunnett's multiple comparison test. Result* p < 0.05* < 0.01* < 0.001 were considered as statistically significant.

RESULT

Pharmacognostical study

Percentage yield

Percentage yield of *Aegle marmelos* Linn Sample A (Bark) and Sample B (Seed) was found to be 7.5 % and 76.6 %. Respectively

Physical characteristics and Phytochemical analysis

Physical characteristics revealed the percentage of purity and inorganic content in plant material. Phytochemical analysis revealed the presence of flavanoid and phenolic compound Like Marmelosin, Marmelin, Lupeol, Eugenol, Limonene, Citral, Rutin which are responsible to exhibit Anti-cancer properties.⁽⁶⁾

Pharmacological study

Effect of *Aegle Marmelos*. Linn on MCF-7 (Human Breast Cancer) cell line by MTT assay

The percentage cell viability count of *Aegle Marmelos*. Linn on MCF-7 Human Breast Cancer Cell line was calculated by using following formula (100xControl-Sample/Control).

The ethanolic extract of *Aegle marmelos*. Linn bark exhibited dose-dependent cytotoxic activity in the *in-vitro* MTT assay. With an IC₅₀ value of 82.17 µg/ml, the ethanol extract demonstrated moderate anti-cancer action at a dose of 100 µg/ml, reducing cell viability to 45.18%. With an IC₅₀ of 218.4 µg/ml, the aqueous extract of *Aegle marmelos*. Linn seed demonstrated low potency and lower action, reducing cell viability only to 74.13%. Control group again has the highest OD.

5FU shows consistently the lowest OD across concentrations, confirming strong cytotoxicity. Aqueous extract consistently shows higher OD than 5FU but lower than the ethanol extract - again suggesting it's more effective than ethanol extract.

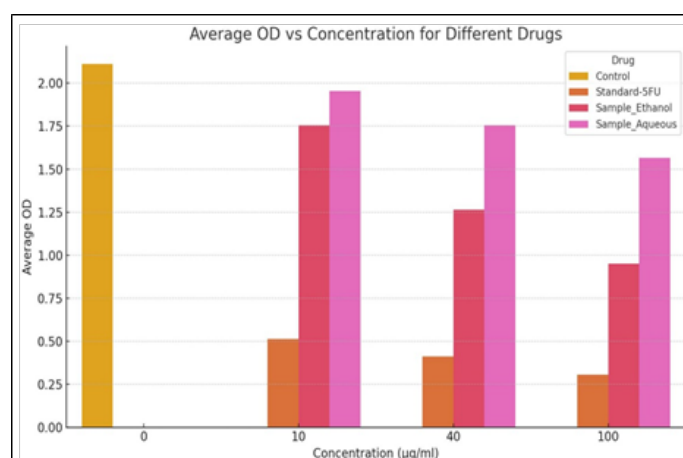


Figure 1: % Cell Viability (MCF-7 cell line) by MTT assay.

Effect of *Aegle Marmelos* Linn. on MCF-7 (Human Breast Cancer) cell line by SRB assay

The ethanolic extract of *Aegle marmelos*. Linn bark had greater cytotoxic potential in the *in-vitro* SRB assay, lowering cell viability from 64.75% at 10 µg/ml to 31.56% at 100 µg/ml, with an IC₅₀ of 44.03 µg/ml. The aqueous extract of *Aegle marmelos*. Linn seed, on the other hand, showed moderate cytotoxicity with an IC₅₀ value of 89.14 µg/ml, demonstrating the greater extraction efficiency of ethanol in cytotoxic phytoconstituents. Control group shows the highest OD at 0 µg/ml, indicating maximum cell viability. All treatments (5FU, ethanol, aqueous) reduce OD as concentration increases, suggesting

Table 1: Effect of Sample A and Sample B on MCF-7 cell line by MTT Assay.

Drug	Concentration	OD1	OD2	OD3	Average OD	% Cell viability	IC50 (µg/ml)
Control	0	2.107	2.114	2.110	2.110		
Standard-5FU	10	0.512	0.513	0.515	0.513	24.32	6
Standard-5FU	40	0.412	0.412	0.412	0.412	19.55	6
Standard-5FU	100	0.306	0.307	0.303	0.305	14.46	6
Sample Ethanol	10	1.756	1.758	1.752	1.755	83.19	82.17
Sample Ethanol	40	1.263	1.266	1.264	1.264	59.92	82.17
Sample Ethanol	100	0.951	0.953	0.950	0.951	45.18	82.17
Sample Aqueous	10	1.956	1.955	1.954	1.955	92.65	218.4
Sample Aqueous	40	1.757	1.755	1.754	1.755	83.14	218.4
Sample Aqueous	100	1.563	1.566	1.564	1.564	74.13	218.4

Table 2: Effect of Sample A and Sample B on MCF-7 cell line by SRB Assay.

Drug	Concentration	OD1	OD2	OD3	Average OD	% Cell viability	IC50
Control	0	2.356	2.355	2.351	2.354		
Standard-5FU	10	1.256	1.253	1.257	1.255	53.32	5.70
Standard-5FU	40	0.756	0.752	0.751	0.753	31.98	5.70
Standard-5FU	100	0.526	0.522	0.523	0.524	22.24	5.70
Sample Ethanol	10	1.526	1.523	1.524	1.524	64.75	44.03
Sample Ethanol	40	1.114	1.112	1.116	1.114	47.32	44.03
Sample Ethanol	100	0.745	0.742	0.742	0.743	31.56	44.03
Sample Aqueous	10	1.922	1.926	1.923	1.924	81.71	89.14
Sample Aqueous	40	1.512	1.513	1.513	1.513	64.27	89.14
Sample Aqueous	100	1.112	1.112	1.114	1.113	47.26	89.14

dose- dependent cytotoxicity. 5FU shows the greatest reduction in OD, indicating the strongest anti-proliferative effect. Aqueous extract is generally more effective than the ethanol extract but less than 5FU.

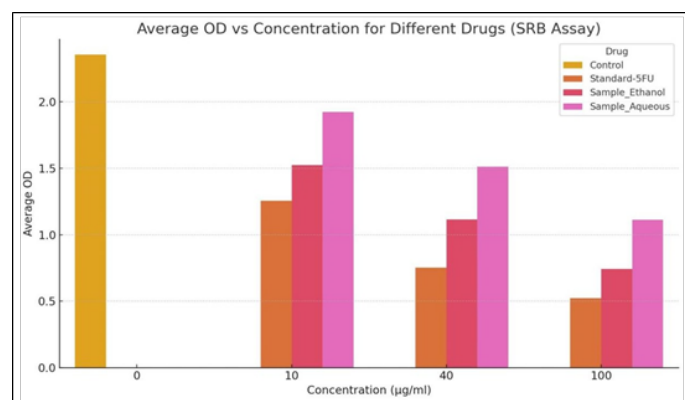


Figure 2: % Cell Viability (MCF-7 cell line) by SRB assay.

CONCLUSION

Aegle marmelos. Linn bark and seeds, a plant material was tested for its anti-cancer activity using conventional cytotoxic tests on MCF-7 human breast cancer cell lines. The ethanolic extract showed dose-dependent cytotoxic activity, reducing cell viability to 45.18% at a dose of 100 µg/ml. The aqueous extract showed low potency and lower action, reducing cell viability to 74.13%. The ethanolic extract had greater cytotoxic potential in the SRB assay, lowering cell viability to 31.56% at 100 µg/ml. The study suggests that the ethanolic extract of *Aegle marmelos*. Linn bark may be a potential anti-cancer agent against MCF-7 cell line.

ACKNOWLEDGEMENT

The authors are grateful to all the direct and indirect hands that has helped us during every step of study.

ABBREVIATIONS

MCF-7- Michigan Cancer Foundation-7, **MTT**: 3-(4,5-Dimethylthiazol-2-yl)-2,5- Diphenyltetrazolium Bromide, **SRB**: Sulforhodamine B, **IARC**: International Agency for Research on cancer.

SUMMARY

This study investigated the anti-cancer properties of *Aegle marmelos* Linn. seeds and bark extracts against human breast cancer cells (MCF-7). The extracts were rich in phytochemicals like phenolic compounds and flavonoids, showed significant cytotoxic activity with a dose-dependent decrease in cell viability. The ethanolic extract of *Aegle marmelos* bark exhibited potent anti-cancer effects, compare to the standard chemotherapeutic drug 5-Fluorouracil. These findings suggest that *Aegle marmelos* Linn. has potential as a plant-based cancer treatment, warranting further research to develop new therapeutic agents.

REFERENCES

1. Mitra S, et al. Cancer and noncoding RNAs. Sci Direct. 2018.
2. Kapse SN, et al. Assessment of *Dolichandrone falcata* Seem. leaves for anti-cancer potential in experimental animal models.

Indian J Pharm Educ Res. 2024 Apr 1;58(2):519-25.

3. Mehraban F, et al. Anti-cancer activity of *Astragalus ovinus* against DMBA-induced breast cancer in rats. *Avicenna J Phytomed.* 2020;10(5):533-45.
4. Bag S, et al. Bio-organic chemistry of natural enediyne anticancer antibiotics. *Bioorg Chem.* 2014.
5. Shakyawar S, et al. Preliminary qualitative phytochemical, physico-chemical and phyto-pharmacognostical standardisation evaluation of the pulp (without seed), pericarp, leaves and seed of *Aegle marmelos* L. (Bael). *Int J Pharm Sci Res.* 2020;11(7):3489-506.
6. Khandelwal KR. *Practical pharmacognosy: techniques and experiments.* 19th ed. Pune: Nirali Prakashan; 2008.
7. Sawant VJ, et al. Comparison of drug delivery potentials of surface functionalized cobalt and zinc ferrite nanohybrids for curcumin into MCF-7 breast cancer cells. *J Magn Magn Mater.* 2016;417:222-9.
8. Manikyam HK, Patil SB. Anti-cancer activity of novel terpenoid, saponin of *Psidium guajava* on MCF-7 cancer cell line using DAPI and MTT assays. *Afr J Pharm Pharmacol.* 2021;15(12):206-11.