Orginal Research

Assessment of the Anticancer Activity of *Aegle marmelos* Leaf Extracts Against Ehrlich's Ascites Carcinoma (EAC) Cell Line in Swiss Albino Mice

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Abstract: Cancer continues to pose a major global health challenge, thereby driving the need for safer and more effective treatment options. This study evaluates the anticancer properties of *Aegle marmelos* leaf extracts against Ehrlich Ascites Carcinoma (EAC) cells in Swiss Albino mice. Three extracts methanolic (APME), chloroform (APCE), and n-hexane (APHE) were prepared and administered intraperitoneally at doses of 50 mg/kg and 100 mg/kg over six consecutive days. The anticancer effects were evaluated based on the viable tumor cell count, percentage (%) inhibition of cell growth, and changes in body weight of the mice that had been treated with extracts. Among the extracts, APHE has shown the highest tumor growth inhibition (62.2% at 100 mg/kg), followed by APME (59.25%) and APCE (54.86%), compared with the standard chemotherapy drug doxorubicin (81.6% inhibition at 0.8 mg/kg/day). Mice treated with the extracts experienced less weight loss than the control group, with an approximate effect of 0.50 +/- 0.06 g/day. A Brine Shrimp Lethality Bioassay demonstrated a dose-dependent cytotoxic effect, with LC50 values of 18.38, 36.79, and 46.45 microgram per milliliter for n-hexane, methanolic, and chloroform extracts, respectively. The tumor-suppressing and cytotoxic effects of *Aegle marmelos* extracts are probably due to the bioactive compounds such as flavonoids, alkaloids, and essential oils, these are known for their apoptotic and antiproliferative activities. Although the extracts were not as effective as doxorubicin, their natural origin and ability to reduce cancer-related weight loss suggest a good potential as a complementary or alternative to cancer therapies. Further research, includes that, mechanistic studies and clinical trials are needed to confirm their therapeutic potential.

Keywords: *Aegle marmelos*; Ehrlich's Ascites Carcinoma (EAC); Anticancer activity; Swiss Albino mice; Plant Extracts

1. Introduction

Cancer is a complex condition that is governed by an array of genetic and epigenetic changes that result in excessive cellular proliferation and expansion of cell populations [1,2]. Mutations in tumor suppressor genes [3], oncogenes [4], and microRNA genes [5] are frequently associated with its progression. As a major public health concern, cancer remains a significant challenge in medical research and treatment [6,7]. Conventional treatments such as hormone therapy [12], radiation [10,11], chemotherapy [8,9], and surgery [13,14], are often used but frequently have major adverse effects. The high mortality rate associated with cancer, along with the adverse effects of anticancer drugs, has prompted researchers to explore alternative treatment options with improved efficacy and reduced toxicity [15]. Due to these challenges, scientists are actively investigating natural compounds with potential anticancer properties [16,17]. Numerous plant-derived bioactive compounds, such as terpenoids, phenolic acids, flavonoids, tannins, lignans, quinones, coumarins, and alkaloids, have shown promising antioxidant activity, playing a crucial role in cancer prevention and treatment [18]. Research suggests that antioxidants exhibit anti-inflammatory, antitumor, antimutagenic, and anticarcinogenic properties, contributing to the inhibition of cancer cell growth and the enhancement of immune function [20,21]. Despite advancements in modern medical science, Ayurvedic treatments

continue to be valued for their therapeutic benefits [22]. Consequently, recent studies have focused on identifying plant-based bioactive compounds with potential anticancer effects [23]. *Aegle marmelos*, also known as Bael, Bengal quince, wood apple, or stone apple, is a medicinal tree that belongs to the Rutaceae family. It is categorized under the order Sapindales and the clades angiosperms, eudicots, and rosids [24,25]. Indigenous to the Indian subcontinent and Southeast Asia, *Aegle marmelos* is widely regarded for its cultural and medicinal significance, particularly in Ayurveda [26,27]. This tropical tree bears fruit with well-documented medicinal properties and is cultivated extensively across Asia. Its fruit, leaves, and bark contain bioactive compounds such as flavonoids, alkaloids, and essential oils, which contribute to its therapeutic potential. Traditionally, different parts of the plant have been used as digestive aids, astringents, antidiabetic agents, and antimicrobial treatments [28]. It has also been employed in the management of gastrointestinal disorders [29], respiratory conditions like asthma and bronchitis [30,31], and chronic illnesses such as diabetes, hypertension, arthritis, and skin infections [32]. Recent scientific studies highlight *Aegle marmelos* for its antioxidant [33] and anticancer potential [34], making it a promising candidate for herbal-based cancer treatments. Given its bioactive composition and therapeutic properties, the present study aims to explore the anticancer potential of *Aegle marmelos* leaf extracts using an in vivo mouse model.

Abbreviation

EAC - Ehrlich's Ascites Carcinoma; APME - Methanolic Extract; APCE - Chloroform Extract; APHE - n-Hexane Extract; DMSO - Dimethyl Sulfoxide; PBS - Phosphate-Buffered Saline; RH - Relative Humidity; LC50 - Median Lethal Concentration; HPLC - High-Performance Liquid Chromatography; i.p. - Intraperitoneal; ICDDR'B - International Centre for Diarrhoeal Disease Research, Bangladesh; SEM - Standard Error of the Mean.

2. Materials and Methods

2.1 Chemicals and reagents

All chemicals and reagents used in this study were of analytical grade and sourced from reputable suppliers. Doxorubicin, obtained from Beacon Pharmaceutical Limited, Bangladesh, served as the standard chemotherapeutic drug and was prepared in distilled water at a concentration of 0.075 mg/ml for intraperitoneal administration. Methanol, chloroform, and n-hexane (HPLC grade) were procured from Sigma-Aldrich (USA) for leaf extract preparation. Dimethyl sulfoxide (DMSO) (Merck, Germany) was used at a 2% concentration as a solvent for dissolving the extracts in experimental treatments. Trypan blue dye (0.4%) was utilized for cell viability assays to differentiate between viable and non-viable Ehrlich's Ascites Carcinoma (EAC) cells. Normal saline solution (0.98% NaCl) from Beximco Pharmaceuticals Ltd, Bangladesh, was prepared for diluting tumor cell suspensions before transplantation. Cotton and Whatman No. 1 filter paper was used for extract filtration, while phosphate-buffered saline (PBS) was employed for cell washing and other experimental procedures. All solutions were freshly prepared before use and stored under appropriate conditions to maintain stability and effectiveness.

2.2 Extract preparation

To remove dirt, collected fresh leaves of the *Aegle marmelos* were washed with cold and then hot water to remove any kind of organism. The leaves were dried in a shaded area for 15 days (at room temperature, 25 ± 2 °C) to prevent photo-oxidation of bioactive components and then ground into a fine powder. The leaf's fine powder (500 g) was extracted by using APME (methanolic), APCE (chloroform), and APHE (n-hexane) at room temperature (25 ± 2 °C) for 15 days with agitation three times a day. Finally, filtration was performed by using cotton and then no.1 Whatman filter paper. The filtrate is then concentrated by using a rotary evaporator and finally air dried. The extract yield was 6.78% (w/w) with methanol, 7 % (w/w) with chloroform, 7.8% (w/w) with n-hexane.

2.3 Experimental animal

Swiss Albino mice, aged 5-7 weeks and weighing between 20-26 grams, were obtained from the Department of Pharmacy, Jahangirnagar University, Dhaka, Bangladesh. These mice were kept under standard environmental conditions in the Molecular Biology lab, with a relative humidity (RH) of 55 ± 5% and a temperature of 22 ± 2°C. Prior to the experiment, the mice were allowed to acclimate for one week in the animal house under a 12-hour light/12-hour dark cycle. These mice have a typical lifespan of 2-3 years, with the potential to live up to 4 years. They were housed in iron cages with sawdust and straw bedding, which was replaced weekly. The mice were fed a standard diet formulated by ICDDR'B, along with an ample supply of water. The animal house maintained a room temperature of 25-32°C, with a controlled light cycle of 14 hours of daylight and 10 hours of darkness. The pellet diet provided by ICDDR'B in Dhaka contained the following nutrient composition per 100 grams: 66 grams of starch, 20 grams of casein, 8 grams of fat, 2 grams of standard vitamins, and 4 grams of salt. The experimental animal is shown in Figure 1

Figure 1: Swiss Albino Mice**,** this figure shows the Swiss Albino mice used in the experiment to assess the anticancer effects of *Aegle marmelos* leaf extracts against Ehrlich's Ascites Carcinoma (EAC). The mice were maintained under controlled conditions with a standard diet before tumor inoculation and treatment.

2.4 Ehrlich's ascites carcinoma (EAC) cell line

Experimental tumors serve as essential models in cancer research, providing valuable insights into tumor growth, progression, and response to therapies. Among these, Ehrlich Ascites Carcinoma (EAC) stands out as one of the most widely used tumor models. EAC cells have been extensively utilized in cancer studies worldwide due to their high proliferative capacity, adaptability, and ability to mimic various aspects of tumor behavior. Ehrlich Ascites Carcinoma was first identified by the renowned scientist Paul Ehrlich in 1907 when he discovered the tumor in the mammary gland of a white mouse. This discovery laid the foundation for further research into experimental tumors. Over time, the present form of EAC cells was developed by researchers Loewenthal and John, who

derived them from various mammary gland-originated cell lines, thereby refining the model for scientific applications.

EAC cells are characterized by a thin yet structurally robust outer membrane, supported by a strong membrane matrix. Studies examining the morphology of both normal and cancerous cells have established that intracellular and surface membranes adhere to the 'unit membrane' structure. This fundamental structure consists of a bimolecular lipid leaflet, lined with proteins or polysaccharides on both sides, playing a crucial role in maintaining cell integrity and function. Like other cancer cells, EAC cells exhibit this unit membrane organization, which contributes to their resilience and rapid proliferation.

In experimental settings, EAC tumors can be propagated in two distinct forms: solid and ascitic. The solid form is cultivated subcutaneously, while the ascitic form is induced by injecting a tumor cell suspension directly into the peritoneal cavity of a mouse. The ascitic tumor manifests as a milky white fluid containing numerous large, rounded tumor cells, which multiply rapidly. A single million EAC tumor cells can proliferate to approximately 25–100 million cells per milliliter within days. Mice carrying such tumors typically survive for 14–30 days, depending on tumor load and host resistance. This short survival period makes EAC a suitable model for studying tumor growth dynamics, drug efficacy, and immune system interactions in cancer research.

2.5 Microscope

The samples loaded on the hemocytometer were seen using a binocular microscope at magnifications of 10x, 40x, and 100x. At 10x low power was used for the overall examination of the distribution of cells, allowing the identification of specific areas of interest and an initial estimate of cell density. By increasing the magnification to 40x, more intricate cellular characteristics were visible, making it easier to observe the arrangements and forms of cells that are essential for differentiating between various cell types. Finally, the 100x magnification provided a highly detailed view that revealed subtle morphological characteristics essential for confirming cell identities. This tiered approach ensured efficient identification, accurate counting, and a comprehensive analysis of the sample.

2.6 Haemacytometer

The haemocytometer is one of the most widely used, low-cost, and effective tools in accurately determining the number of cells in samples. This is an inexpensive yet specialized device perfect for microscopic counting of cells and provides an accurate assessment of the cell concentration in many different biological and medical applications [35]. A haemocytometer consists of a thick glass slide with two designated counting chambers, each systematically divided into nine large squares, each measuring 1 mm^2 in area. These chambers are on a surface etched and silvered, separated by a central groove used to reinforce the structural integrity of the slide and become a means for easy loading of the cell suspension [36,37]. To ensure accurate cell counting, a coverslip is placed on top of the raised supports of the 'H'-shaped troughs, enclosing both counting chambers and ensuring a uniform sample distribution. Each counting chamber is further equipped with a 'V-shaped notch or an indentation at either end, where the prepared cell suspension is carefully introduced into the chamber [38]. Once the cell suspension is loaded, it spreads evenly within the chamber, filling a precisely defined volume of liquid. The engraved grid on the surface of the counting chamber ensures an organized and systematic approach to counting, preventing any errors or discrepancies. The haemocytometer is then placed on the microscope stage, and the cell suspension is observed at an appropriate magnification to facilitate counting [39]. By carefully analyzing the number of cells within the designated grid areas and applying the necessary dilution factors, researchers can accurately determine the cell concentration per unit volume. The subsequent cell concentration per ml was determined using the following procedure.

Number of cells per ml=

$$\frac{\text{The average count per square} \times \text{Dilution factor}}{\text{Depth of fluid under cover slip} \times \text{Area counted.}}$$

$$\frac{\text{The average count per square} \times \text{Dilution factor}}{0.1\ \text{mm} \times (1\ \text{mm})^2}$$

$$\frac{\text{The average count per square} \times \text{Dilution factor}}{0.1 \times (1\ \text{mm})^3}$$

$$\frac{\text{The average count per square} \times \text{Dilution factor}}{0.1 \times 10^{-3}\ \text{ml}}$$

$$\frac{\text{The average count per square} \times \text{Dilution factor}}{0.1 \times 10^{-3}\ \text{ml}}$$

$$\frac{\text{The average count per square} \times \text{Dilution factor} \times 10^4}{\text{Depth of fluid under cover slip} \times \text{Area counted}}$$

2.7 Preparation of stock solution for the test samples

The preparation of stock solutions for the test samples involved dissolving *Aegle marmelos* leaf extracts in a solvent to ensure proper administration and bioavailability in the experimental animals. Three extracts—methanolic (APME), chloroform (APCE), and n-hexane (APHE)—were prepared using 2% Dimethyl Sulfoxide (DMSO), selected for its ability to dissolve a wide range of organic compounds and its compatibility with biological systems at low concentrations. Each extract was dissolved in DMSO to create a stock solution with a concentration of 100 mg/mL, which was then diluted with phosphate-buffered saline (PBS) to achieve working concentrations of 50 mg/kg and 100 mg/kg for intraperitoneal (i.p.) administration. For instance, a 20-gram mouse received 0.1 mL for a 50 mg/kg dose and 0.2 mL for a 100 mg/kg dose. In addition, doxorubicin, used as the standard chemotherapeutic agent, was dissolved in distilled water at 0.075 mg/mL and administered at a dose of 0.8 mg/kg/day i.p. The control group was given a solution containing 2% DMSO in PBS, ensuring that any observed effects were attributable to the extracts rather than the solvent. All stock solutions were freshly prepared before each administration to maintain stability and efficacy, stored at 4°C when not in use, and brought to room temperature before administration, thereby ensuring uniform dosing and accurate assessment of the anticancer effects of the extracts.

2.8 Experimental tumor model

The Ehrlich Ascites Carcinoma (EAC) cell line used in this study was initially obtained from the Department of Biochemistry and Molecular Biology, University of Rajshahi, Bangladesh. To ensure a continuous and viable supply of tumor cells for experimental use, the culture and maintenance of EAC cells were carried out following the established protocol described by Alam et al. [40], with slight modifications tailored to the specific laboratory conditions. For propagation, EAC cells were maintained in Swiss Albino mice through serial intraperitoneal (i.p.) transplantation. Every week, freshly isolated EAC cells were collected from a donor mouse that had been inoculated with tumor cells six to seven days prior. The ascitic fluid containing tumor cells was carefully withdrawn using a sterile syringe and subsequently diluted with normal saline solution (1% NaCl) to ensure an optimal cell concentration for transplantation. To assess cell viability and facilitate the separation of macrophages from tumor cells, the diluted cell suspension was placed in a sterile petri dish and incubated at 37.5°C in an air incubator for one hour.

During the incubation period, macrophages adhered firmly to the bottom surface of the culture vessel, allowing them to be distinguished from the free-floating tumor cells. This selective adherence facilitated the effective separation of macrophages from the EAC cells, ensuring a purer tumor cell suspension for subsequent experiments. After incubation, the petri dish was gently agitated using a brief vortexing step to resuspend any loosely attached tumor cells. The supernatant,

now enriched with EAC cells, was carefully collected using a sterile pipette and transferred to a fresh container for further processing. To standardize the tumor cell concentration for experimental use, the collected suspension was carefully adjusted to approximately 2×10^6 cells/ml. This was achieved using a hemocytometer, a specialized counting chamber that provides an accurate measurement of cell density. To ensure the viability of the tumor cells, a 0.4% trypan blue dye exclusion assay was performed. Throughout the transplantation process, strict aseptic conditions were maintained to ensure accuracy and reproducibility.

2.9 Transplantation of ascitic tumor

Ascitic fluid was drawn out from different tumor-bearing Swiss albino mice at the respective log phases of tumor cells. A 3ml syringe filled with a 20-gauge needle was used for this tumor cell aspiration. The freshly drawn fluid was diluted with normal saline (0.98% NaCl solution) and the tumor cell number was adjusted to approximately 2106 cells/ ml by counting the cell number with the help of a haemocytometer. The viability of tumor cells was observed by trypen blue dye (0.4%) exclusion assay. Cell samples showing above 90% viability were used for transplantation. Tumor suspension of 0.1 ml was injected intraperitoneally (i. p.) into each Swiss albino mouse (Figure 2). The strict aseptic condition was maintained throughout the transplantation process. *In vivo,* the antitumor activity of the test samples was determined by measuring the effect of samples on tumor cell growth of EAC cell-bearing mice.

Figure 2: Transplantation of Ascitic Tumor, this figure depicts the intraperitoneal injection of Ehrlich's Ascites Carcinoma (EAC) cells into Swiss Albino mice. The tumor cell suspension was prepared under aseptic conditions, diluted with saline, and adjusted to 2×10^6 cells/mL. Trypan blue dye confirmed cell viability before transplantation, enabling the in vivo assessment of *Aegle marmelos* leaf extracts' anticancer effects.

2.10 Studies on EAC Cell Growth Inhibition

To determine the cell growth inhibition of the leaf extracts five groups of Swiss albino mice (6 in each group) weighing 20-25 gm were used. For therapeutic evaluation 136×10^4 EAC cells in every mouse were inoculated into each group of mice on day "0". Treatments were started after 24 hours of tumor inoculation and continued for six days. Group one to three or every APME (methanolic), APCE (chloroform), and APHE (*n*-hexane) received the test compound at the doses of 50 mg/kg and 100mg/kg/day (i. p). The standard group received Doxorubicin at the dose of 0.8 mg/kg/day (i. p) and group five was used as a control. On day six, the mice in each group were sacrificed, and the total

intraperitoneal tumor cells were collected using 0.98% normal saline. Viable cells were first identified by using trypen blue and then counted by a haemocytometer. The total number of viable cells in every animal of the treated groups was compared with those of control (EAC treated only).

2.11 Evaluation of Weight Loss

To assess weight loss, the weight of each mouse was measured daily for six consecutive days following the inoculation of carcinoma cells, using an electric balance (KAMRY, electronic kitchen scale, China) during the treatment period. The rate of weight loss was then calculated by taking the average weight values for both the control and treated mice, using the following equation:

The rate of weight loss

$$= \frac{(\text{1st day weight} - \text{Last day weight})}{\text{Total day of treatment}} \text{ gm/day}$$

2.12 Brine Shrimp Toxicity Assessment

The Brine Shrimp Toxicity Bioassay was conducted following a conventional method with minor modifications. *Artemia salina* nauplii was used as the test organism for this study. For the experiment, 16 mg of the plant extract was dissolved in 250 μL of pure dimethyl sulfoxide (DMSO) and diluted to a final volume of 25 mL with seawater, obtaining a stock solution with a concentration of 900 μg/mL. Serial dilutions were performed to prepare solutions of varying concentrations (200, 150, 100, 75, 50, 25, 12.5, and 6.25 μg/mL). Each pre-marked vial contained 2.5 mL of seawater, to which 2.5 mL of the respective plant extract solution was added, making a final volume of 5 mL. Ten live brine shrimp nauplii were introduced into each vial. The experiment was conducted twice, and after 24 hours, the number of surviving nauplii was counted using a magnifying glass and recorded for analysis.

3. Results

3.1 EAC cell growth inhibition

The in vivo antitumor activity of different extracts—methanolic (APME), n-hexane (APHE), and chloroform (APCE)—from *Aegle marmelos* leaves was evaluated in EAC cell-bearing mice. The assessment was based on viable EAC cell count and percentage inhibition of cell growth. In the untreated EAC control group, the average number of viable tumor cells per mouse was recorded as $(10.12 \pm 2.72) \times 10^7$ cells/ml. Treatment with Doxorubicin (0.8 mg/kg/day) resulted in the most significant reduction in viable cells, with an EAC cell growth inhibition of 81.6%, showing a viable cell count of $1.87 \pm 0.05 \times 10^7$ cells/ml.

Treatment with APME at doses of 50 mg/kg and 100 mg/kg significantly reduced the viable cell count ($P<0.05$). APME exhibited 50.31% and 59.25% EAC cell growth inhibition at 50 mg/kg and 100 mg/kg, respectively, with viable cell counts of 3.95 ± 1.87 and $3.20 \pm 2.06 \times 10^7$ cells/ml. Similarly, APCE demonstrated 30.21% and 54.86% inhibition at 50 mg/kg and 100 mg/kg, respectively, with viable cell counts of 6.2 ± 2.25 and $3.90 \pm 1.56 \times 10^7$ cells/ml. In comparison, APHE showed the highest inhibition among the extracts, with 50.60% and 62.2% EAC cell growth inhibition at 50 mg/kg and 100 mg/kg, respectively, with viable cell counts of 3.68 ± 1.80 and $2.3 \pm 1.34 \times 10^7$ cells/ml.

The order of EAC cell growth inhibition by different treatments was as follows: Doxorubicin > APHE > APME > APCE. The results of growth inhibition are presented in Table 1, and the graphical representation is shown in Figure 3.

Table 1: Effect of *Aegle marmelos* leaf extracts on Ehrlich ascites carcinoma (EAC) cell growth

Name of Exp.	Nature of the drug	Dose in mg/kg/day (i.p)	No of EAC cells in mice on day 6 after tumor cell inoculation (x 10^7 cells/ml)	% Of cell growth inhibition
Control (EAC cell-bearing mice)		----	10.12±2.726665	---
Doxorubicin	Standard	0.8 mg/kg	1.87±.05	81.6
APME	Experimental	50 mg/kg	3.95±1.87	50.31
		100 mg/kg	3.20±2.06	59.25
APCE	Experimental	50 mg/kg	6.2±2.25	30.21
		100 mg/kg	3.90±1.56	54.86
APHE	Experimental	50 mg/kg	3.68±1.80	50.60
		100 mg/kg	2.3±1.34	62.2

Number of mice in each Cage (n=6); the results were shown as Mean ± SEM, where significant values are P<0.05 for 50mg/kg body weight and for 100mg/kg body weight significant values are p<0.01.

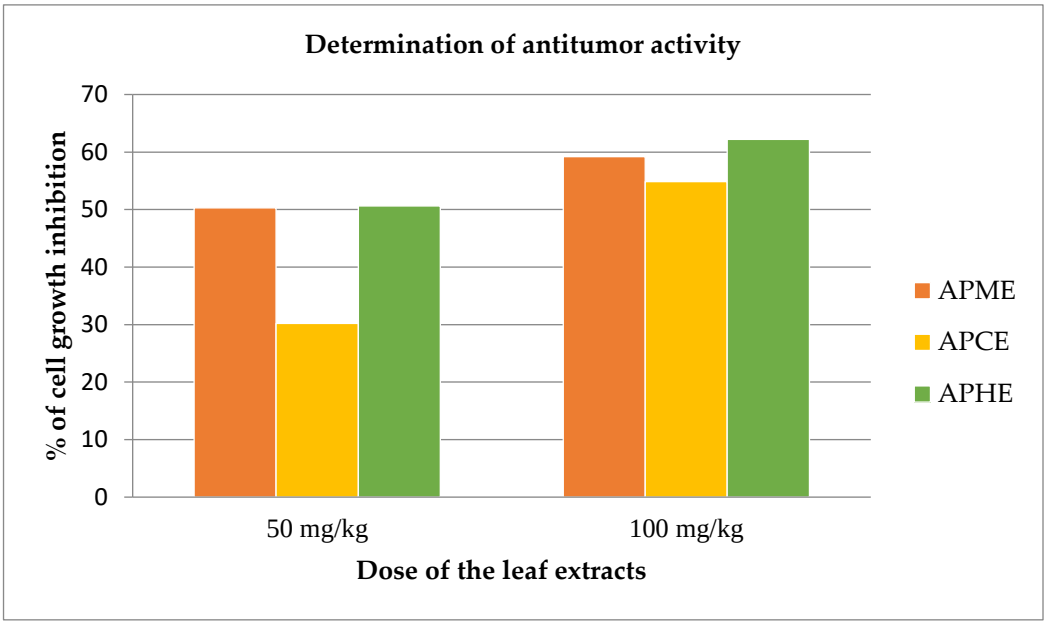

Figure 3: Determination of Antitumor Activity of Aegle marmelos Leaf Extracts, this figure presents the percentage inhibition of Ehrlich's Ascites Carcinoma (EAC) cell growth in Swiss Albino mice treated with different *Aegle marmelos* leaf extracts (APME, APCE, and APHE) at 50 mg/kg and 100 mg/kg doses. The results are compared to the standard chemotherapeutic drug doxorubicin (0.8 mg/kg/day). Among the extracts, APHE exhibited the highest tumor growth inhibition (62.2% at 100 mg/kg), followed by APME and APCE, while doxorubicin showed the highest inhibition (81.6%). The figure visually represents the efficacy of these extracts in suppressing tumor growth.

3.2 Evaluation of Weight Loss

Weight loss is a common symptom of cancer. In the experiment, the mice gradually lost weight after the inoculation of cancer cells. The rate of weight loss was 1.3 ± 0.08 g/day for the control mice and approximately 0.50 ± 0.06 g/day for the leaf-treated mice. The leaf-treated mice showed a lower rate of weight loss compared to the control mice exhibited the highest rate of weight loss, as depicted in Figure 4.

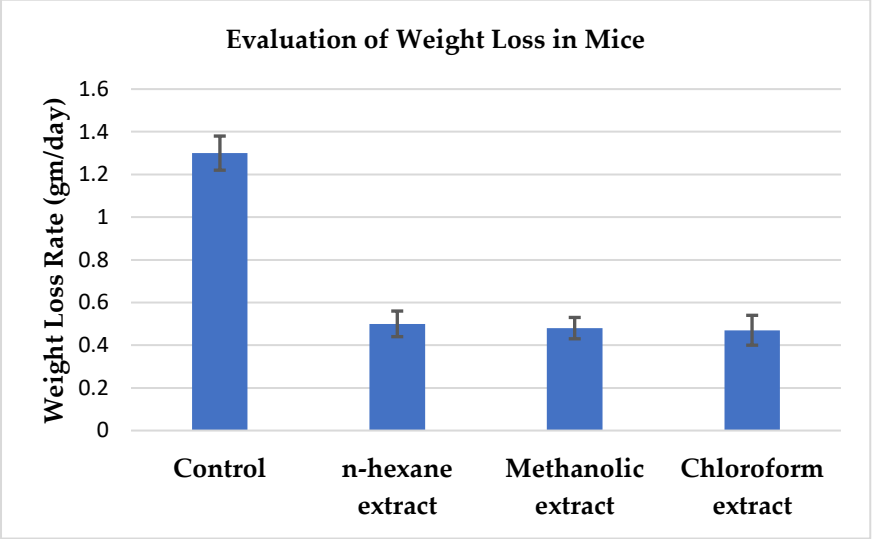

Figure 4: Weight loss evaluation in Swiss Albino mice following treatment with *Aegle marmelos* leaf extracts. Data are presented as mean ± SD (n = 6) for all tested doses. Significant differences from the control group are indicated, with statistical significance levels set at $p < 0.05$ and $p < 0.001$.

3.3 Brine Shrimp Toxicity Assessment

The cytotoxic potential of methanolic, n-hexane, and chloroform extracts of *Aegle marmelos* leaves was assessed using the brine shrimp lethality assay. The results revealed significant toxicity in a dose-dependent manner. At a concentration of 70 µg/mL, the methanolic extract induced 100% mortality. The LC50 values, calculated from the best-fit line slope, were 18.38, 36.79, and 46.45 µg/mL for n-hexane, methanolic, and chloroform extracts, respectively. The pronounced lethality of *Aegle marmelos* leaf extracts indicate the presence of potent cytotoxic compounds, highlighting the need for further investigation into their potential anticancer properties.

4. Discussion

This study evaluated the anticancer potential of *Aegle marmelos* leaf extracts against Ehrlich's Ascites Carcinoma (EAC) in Swiss Albino mice, revealing significant tumor growth inhibition and weight-preserving effects. Among the extracts, APHE (n-hexane extract) demonstrated the highest inhibition of EAC cell growth (62.2% at 100 mg/kg), followed by APME (methanolic extract) and APCE (chloroform extract). These results suggest that *Aegle marmelos* contains bioactive compounds with potent anticancer properties, potentially mediated through apoptosis induction, disruption of tumor cell proliferation, and modulation of oxidative stress. Aside from the suppression of tumor growth, weight loss is assessed to determine the progression of cancer and the effectiveness of treatment. Cancer-related cachexia is a serious condition accompanying significant weight loss and loss of muscle mass, which greatly influences patient survival. The findings showed that untreated EAC-bearing mice exhibited a notable weight loss (1.3 ± 0.08 g/day), whereas extract-treated mice, had a lower weight reduction (0.50 ± 0.06 g/day). This suggests that *Aegle marmelos* extracts may not

only inhibit tumor growth but also help mitigate cancer-related cachexia, potentially improving overall health and survival.

The cytotoxic potential of the extracts was further confirmed using a Brine Shrimp Lethality Bioassay. The LC50 values were determined to be 18.38, 36.79, and 46.45 μg/mL for the n-hexane, methanolic, and chloroform extracts, respectively. This supports the presence of bioactive compounds that may contribute to their anticancer activity. While the standard chemotherapeutic agent doxorubicin exhibited the highest tumor growth inhibition (81.6%), it is associated with severe side effects such as cardiotoxicity and immunosuppression. In contrast, *Aegle marmelos* extracts offer a natural and potentially safer alternative, highlighting their value in complementary or alternative cancer therapy. In light of the evidence supportive of these findings, other limitations have to be addressed. The precise molecular mechanisms through which *Aegle marmelos* elicits its anticancer effects are still not understood. Future work should focus on establishing these mechanisms, identifying the specific active compounds or phytochemical constituents, or conducting a histopathological analysis. Long-term toxicity and safety tests and clinical trials are also essential to affirm their potential as cancer treatments in humans.

Overall, the findings of this study provide compelling evidence that Aegle marmelos leaf extracts have considerable anticancer potential and reduce cancer-associated weight loss. These results lend credence to further research into their potential as a natural, effective, and safer alternative for cancer posttherapy.

5. Conclusion

This study demonstrates the significant anticancer potential of *Aegle marmelos* leaf extracts against Ehrlich's Ascites Carcinoma (EAC) in Swiss Albino mice. Among the tested extracts, the n-hexane extract (APHE) exhibited the highest tumor growth inhibition (62.2% at 100 mg/kg), followed by the methanolic (APME) and chloroform (APCE) extracts. Additionally, extract-treated mice showed reduced weight loss, suggesting a potential role in mitigating cancer-associated cachexia. Although the extracts displayed slightly lower efficacy compared to the standard chemotherapeutic agent doxorubicin (81.6% inhibition), their natural origin and lower toxicity highlight their potential as safer alternatives. Certain bioactive compounds including flavonoids, alkaloids, and essential oils can probably contribute to the anticancer effects via apoptosis induction and tumor growth inhibition. While these indications are encouraging, further studies are needed to elucidate the precise molecular mechanisms involved, optimize dosage, and evaluate safety in the long run. Clinical trials will be essential to validate the therapeutic potential of *Aegle marmelos* as a complementary or alternative anticancer treatment.

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