MEDFARM: Jurnal Farmasi dan Kesehatan, Vol. 15, No. 1, 2026, Page 101-116
e-ISSN : 2715-9957
p-ISSN: 2354-8487

Phytochemical Profile, Antioxidant Activity, and Cell Viability of *Aegle marmelos* Fruit Pulp Ethanolic Extract on SKOV-3 Ovarian Cancer Cells

Sri Wahyuni*[1], E.M. Sutrisna[2], Maryati[3], Sari Haryanti[4], Rona Nasywa Mahira[5]

[1)] Program of Doctoral in Pharmacy, Faculty of Pharmacy, Universitas Muhammadiyah Surakarta, Indonesia
[2)] Department of Biomedicine, Faculty of Medicine, Universitas Muhammadiyah Surakarta, Indonesia
[3)] Center for Cancer Epidemiology Studies, Faculty of Medicine, Universitas Muhammadiyah Surakarta, Indonesia
[4)] Research Center for Pharmaceutical Ingredient and Traditional Medicine, National Agency of Research and Innovation, Cibinong, Bogor Regency, West of Java, Indonesia
[5)]Medical Education Study Program, Faculty of Medicine, Muhammadiyah University of Surakarta, Indonesia

e-mail:
[1)] sw224@ums.ac.id
[2)] es233@ums.ac.id

ABSTRACT

Ovarian cancer remains a leading cause of cancer related mortality among women, emphasizing the need for safer more effective therapeutic strategies. Natural products are increasingly explored due to their diverse bioactive compounds. This study evaluated the phytochemical profile, total phenolic content (TPC), antioxidant activity, and cytotoxic effects of the ethanolic extract of *Aegle marmelos* fruit pulp on SKOV-3 ovarian cancer cells. Phytochemical screening was conducted using standard qualitative methods. TPC was determined by Folin-Ciocalteu Assay, antioxidant activity by the DPPH method, and cytotoxic using MTT assay. The extract contained flavonoids, alkaloids, tannins, and triterpenoids. The TPC was 8.22 mg GAE / g extract, indicating relatively low phenolic content. Consistently, antioxidant activity was week, with an IC_{50} value of approximately 712 ppm. The extract did not significantly reduce SKOV-3 cell viability, which remained above 90% at all tested concentrations. However, morphological changes, including cell shrinkage dan reduce adhesion, were observed at higher concentrations, suggesting sublethal cellular stress responses. In conclusion, the ethanolic extract of *Aegle marmelos* fruit pulp exhibits the presence of bioactive compounds and limited biological activity but lacks strong cytotoxic effects against SKOV-3 cells. These findings indicate its potential role as chemopreventive or supportive agent rather than a primary anticancer compound. Further studies are required to identify active constituents and clarify underlying mechanism.

Keywords : *Aegle marmelos*, Fruit Pulp Extract, Antioxidant Activity, Total Phenolic Content, SKOV-3 Cell Viability

Introduction

Ovarian cancer is widely recognized as one of the most aggressive gynecological malignancies and remains a major contributor to cancer related

mortality among women worldwide. The high fatality rate is primarily attributed to the lack of specific symptoms during early-stage progression, which often results in delayed diagnosis at advanced stages when metastasis has already occurred (Torre et al., 2018). Under such conditions, treatment outcomes are frequently poor due to extensive tumor progression and the development of resistance to conventional chemotherapeutic agents (Vang et al., 2016).

Current therapeutic regimens, particularly platinum-based compounds and taxanes, initially show favorable clinical responses. However, their long-term effectiveness is limited by several challenges, including systemic toxicity, severe side effects, and the emergence of chemoresistance (Vang et al., 2016; Lheureux et al., 2019). These limitations highlight the urgent need for alternative therapeutic strategies, especially those derived from natural sources, which may offer improved safety profiles and potential synergistic effects when combined with standard chemotherapy (Cragg & Pezzuto, 2016).

Natural products have attracted considerable attention as potential anticancer agents due to their structural diversity and ability to modulate multiple molecular targets. One such medicinal plant is *Aegle marmelos*, which has been widely used in traditional medicine across Asia for the treatment of various diseases. This plant exhibits a broad spectrum of pharmacological activities, including antimicrobial, anti inflammatory, antidiabetic, antioxidant, and anticancer properties (Mujeeb et al., 2014; Ahmad et al., 2017; Sukanth et al., 2021). Different parts of the plant, including leaves, bark, roots, and fruits, have traditionally been utilized to manage gastrointestinal disorders, metabolic diseases, and inflammatory conditions (Aziz et al., 2021).

Phytochemical investigations have identified numerous bioactive constituents in *A. marmelos*, such as coumarins, flavonoids, and phenolic derivates including marmelosin, scopoletin, and auraptenin. These compounds have demonstrated cytotoxic activity against various cancer cell lines, including lung, breast, and liver cancers, through mechanisms such as inhibition of cell proliferation, induction of apoptosis, and suppression of metastasis (Akhouri et al., 2020; Sukanth et al., 2021). In addition, these phytochemicals contribute to antioxidant activity, which plays a critical role in regulating oxidative stress, a key factor in carcinogenesis. Excessive production of reactive oxygen species (ROS) can lead DNA damage, genomic instability, and uncontrolled cell proliferation, thereby promoting cancer development (Wang et al., 2022). Therefore, plant derived antioxidants are considered promising candidates for cancer prevention and therapy

Despite extensive studies on the leaves and bark of *A. marmelos*, research focusing on the fruit pulp, particularly its anticancer potential against ovarian cancer models, remains limited. This represents a significant research gap, given that different plat parts may contain distinct phytochemical profiles and bioactivities.

Therefore, this study aims to evaluated the antioxidant activity, total phenolic content (TPC), phytochemical profile, and cytotoxic activity of ethanolic extract of *Aegle marmelos* fruit pulp against SKOV-3 ovarian cancer cells. By integrating phytochemical analysis with biological activity assays, this research is expected to provide scientific evidence supporting the potential use of *A. marmelos* fruit pulp as a

natural source of anticancer agents. Furthermore, this study contributes to the growing body of research exploring plant-based compounds as safer and more effective alternatives or complements to conventional chemotherapy (Wahyuni et al., 2025).

Methodology

Material, Methods and Ethical Approval

Ethical Approval

This study was approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Muhammadiyah Surakarta, with ethical clearance number 5402/A.1/KEPK-FKUMS/XI/2024

Plant Material

The fruit of *Aegle marmelos* was obtained from Materia Medica Batu, Malang, East Java, Indonesia (GPS coordinates : -7.8786^{o} S, 112.5339^{o} E) on February 23, 2024. The plant material was taxonomically identified at the UPT Herbal Laboratory of Materia Medica Batu, with identification number 000.9.3/581/102.20/2024. The fruit pulp was separated, cleaned, dried , and ground into powdered simplicial prior to extraction.

Extraction

Extraction of *Aegle marmelos* fruit pulp was carried out using maceration method with 96% ethanol (Brataco Chemical, Indonesia, analytical grade) as the solvent at a ratio of 1 : 10 (w/v). The powdered simplicial (1kg) was immersed in ethanol and subjected to intermittent stirring for the 6 hours, followed by maceration 18 hours. The processs was continued for up to 5 days to ensure optimal extraction of bioactive compounds. The extract was filtered and concentrated using a rotatory evaporator (Biobase, China) under reduced pressure, followed by further evaporation using water bath (Memmert, Germany) to obtain a viscous ethanolic extract. This method was adapted from standard extraction procedures described in the Indonesian Herbal Pharmacopoeia and previous studies (Kemenkes RI, 2017); Panca et al., 2022; Handayani et al., 2024)

Phytochemical Screening of Ethanolic Extract of *Aegle marmelos* Fruit Pulp

Phytochemical screening of the ethanolic extract of *Aegle marmelos* fruit pulp was performed using standard qualitative methods based on colorimetric and precipitation reactions to identify major classes of secondary metabolites. The procedures were adapted from classical phytochemical methods (Harbone, 1987) and supported by more recent studies (Ijoma et al., 2022).

Alkaloid were identify by dissolving the extract in chloroform followed by the addition of ammonia and sulfuric acid (H_2SO_4). The acidic layer was subsequently treated with Mayer, Wagner, and Dragendorff reagents. The formation of white, reddish-brown, or orange precipitates indicated a positive result.

Flavonoid were detected using the Shinoda test. The extract was boiled in distilled water, filtered, and the filtrate was treated with magnesium powder and

concentrated hydrochloric acid (HCl). The appearance of red, orange, or yellow coloration confirmed the presence of flavonoids.

Saponins were analyzed using foam test. The extract was vigorously shaken with distilled water and a few drops of HCl were added. The formation of stable and persistent foam indicated the presence of saponins.

Tannins and phenolic compounds were identified using ferric chloride ($FeCl_3$) and gelatine test. A greenish-black or bluish-black coloration after $FeCl_3$ addition confirmed tannins, while the formation of white precipitate with gelatine indicated phenolic compounds. Additionally, a ferricyanide-ammonia reaction producing a deep red colour further supported the presence of tannins.

Steroid and triterpenoid were determined using Libermann-Burchard reaction. The extract was treated with chloroform and acetic anhydride, followed by the addition of concentrated sulfuric acid. A blue -green coloration indicated steroids, while a reddish or violet colour suggested triterpenoids.

Total Phenolic Content (TPC) Analysis of Ethanolic Extract of *Aegle marmelos* Fruit Pulp.

The total phenolic content (TPC) of the ethanolic extract of *Aegle marmelos* fruit pulp was determined using the Folin-Ciocalteu method based on the Indonesia Herbal Pharmacopoeia Volume II (Kemenkes RI,2017) and supported by previous studies (Utami et al., 2024; Wahid et al. 2023).

The extract sample or gallic acid standard solution was mixed with Folin-Ciocleu reagent (Merck, Germany; analytical grade; diluted 1 :10 with distilled water) and allowed to stand for 5 minutes at room temperature. Subsequently, 7% sodium carbonate (Na_2CO_3) solution (Merck, Germany) was added to the mixture. The reaction mixture was then incubated at 45°C for 15 minutes or alternatively at room temperature for 30 minutes to allow colour development. The absorbance was measured at wavelength of 765 nm using UV-Vis spectrophotometer (Shimadzu, Japan). A calibration curve was constructed using gallic acid (Sigma-Aldrich, USA; standard grade) at various concentrations. The total phenolic content was calculated from the calibration curve and expressed as milligrams of gallic acid equivalents per gram of dried extract (mg GAE/g extract)

Antioxidant Activity of Ethanolic Extract of *Aegle marmelos* Fruit Pulp Using the DPPH Method

The antioxidant activity of the ethanolic extract of *Aegle marmelos* fruit pulp was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, which measures the ability of the sample to donate hydrogen atoms or electrons to neutralize free radicals (Nurwaini and Fatimah, 2024; Ekasari et al., 2023).

The extract was dissolved in methanol and prepared at various concentrations (62.5, 125, 250, 500, and 1000 ppm). Each sample solution was mixed with 0.004% (v/v) DPPH solution (Sigma-Aldrich, USA) prepared in methanol (Merck, Germany). The mixture was incubated in the dark at room temperature for 30 minutes to allow the reaction to occur.

After incubation, the decrease in absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Shimadzu, Japan). The antioxidant activity was expressed as the percentage of DPPH radical inhibition (%inhibition), calculated using the following equation :

$$\%\ Inhibition = \frac{(A_{control} - A_{sample})}{A_{control}} x\ 100\%$$

When $A_{control}$ is the Absorbance of the control (DPPH solution without sample) and A_{sample} is the absorbance of the test sample.

The IC_{50} value, defined as the concentration of extract required to inhibit 50% of DPPH radicals, was determined from the linier regression equation :

$$Y = bx + a$$

Where Y represent % inhibition and x represent concentration. The IC50 value was calculated using the equation :

$$IC_{50} = \frac{(50 - a)}{b}$$

Where a is the intercept and b is the slope of the regression line.

Cell Viability Assay of *Aegle marmelos* Fruit Pulp Ethanolic Extract on SKOV-3 Cells

The cytotoxic activity of the ethanolic extract of Aegle marmelos fruit pulp was evaluated using the MTT assay on human ovarian cancer SKOV-3 cells. The SKOV-3 cell line was obtained from the European Collection of Authenticated Cell Cultures (ECACC, Sigma-Aldrich, UK).

Cells were seeded into 96-well microplates at a density of 3 x 10^3 cells/well and incubated for the 24 hours at 37°C in a humidified atmosphere containing 5% CO_2 to allow optimal cell attachment and growth. After incubation, the culture medium (McCoy's 5A medium; Sigma-Aldrich, USA) was replaced with fresh medium containing various concentration of ethanolic extract of *Aegle marmelos* fruit pulp. Dimethyl sulfoxide (DMSO) was used as a co-solvent control. The treated cells were further incubated for 48 hours under the same condition (Liu et al., 2019).

Following treatment, the medium was discarded, and the cells were washed with phosphate=buffered saline (PBS; pH 704). Subsequently, 100 µL of fresh medium and 10 µL of MTT solution (5 mg/mL) were added to each well and incubated for 4-6 hours to allow mitochondrial dehydrogenase enzymes in viable cells to reduce MTT into insoluble purple formazan crystals (Maryati et al., 2020; Indrayudha et al., 2022; Wulandari et al., 2024).

The reaction was terminated by adding a stopper solution consisting of 10 % sodium dodecyl sulfate (SDS) in 0.01 N hydrochloric acid (HCl), followed by overnight incubation at room temperature in the dark to ensure complete dissolution of the formazan crystals. Absorbance was measured at 570 nm using a microplate reader. Cell viability was expressed as a percentage relative to untreated control cells, reflecting the metabolic activity of viable cells. This assay is widely used to evaluated cytotoxic effects of natural products through mitochondrial activity as an indicator of cell survival (Maryati et al., 2020; Endah et al., 2022).

Data Analysis

All experimental data were expressed mean ± standard deviation (SD) from three independent replicates. Statistical analysis was performed using JASP software (Version 0.18, University of Amsterdam, Netherlands). Normality of the data distribution was evaluated using the Shapiro-Wilk test, while homogeneity of variances was assessed using Levene's test, since the data did not meet assumptions of normality and homogeneity ($p<0.05$), non parametric statistical analysis was applied. Differences among multiple groups were analyzed using the Kruskal-Wallis test, followed by Dunn's post hoc test with Holm correction to identify pairwise differences between treatment groups. A p-value < 0.05 was considered statistically significant. For antioxidant analysis, liner regression was performed to determine the relationship between extract concentration and percentage inhibition, and to calculate the IC_{50} value.

Result and Discussion

The extraction of *Aegle marmelos* fruit pulp using 98% ethanol produced 488.91 g of extract with a yield of 48.89%. this relatively extract recovery, suggesting that ethanol is an effective solvent for isolating a broad spectrum of phytochemical constituents. This efficiency is likely attributes to the intermediate polarity of ethanol, which enables the dissolution of both polar and semi polar compounds, including phenolic and flavonoids (Rodriguez De Luna at al., 2020). Extraction yield is influenced not only by solvent characteristics but also by the physicochemical properties of the plant matrix and extraction conditions, all of which may contribute to the overall biological activity of the resulting extract (Chatri et al., 2024; Azmir et al., 2013)

Table 1. Phytochemical Screening of Ethanolic Extract of *Aegle marmelos* Fruit Pulp.

Phytochemical Compound	Result
Flavonoids	+
Alkaloids	+
Tannin / Phenolics	+
Steroids	-
Triterpenoids	+
Saponins	-

Note : (+) detected; (-) not detected

Phytochemical screening confirmed the presence of several secondary metabolites, including flavonoids, alkaloids, tannins / phenolics, and triterpenoids (Table 1). These classes of compounds are widely reported to possess diverse biological activities. Flavonoid, for instance, are known to function as potent antioxidants through free radical scavenging mechanisms and have also been associated with antiproliferative and pro apoptotic effects in cancer cells (Panche et al., 2016; Neha et al., 2021). Alkaloids contribute to a wide range of pharmacological effects, including anti inflammatory and cytoprotective activities (Olorokooba et al., 2021). Meanwhile, tannins and other phenolic compounds play an essential role in mitigating oxidative stress through hydrogen donation and metal chelation

(Chandrasekara & Shahidi, 2018). These compounds also possess antimicrobial and wound-healing properties, supporting the traditional use of *Aegle marmelos* in treating infections (Gop et al., 2022; Babu et al., 2022). Triterpenoid have also been implicated in modulating signaling pathwaya associated with inflammation and cancer progression (Murthy et al., 2019). The presence of these bioactive constituents suggests a synergistic interaction that enhances the overall pharmacological potential of *Aegle marmelos* fruit pulp, particularly in relation to its antioxidant and anticancer activities.

Table 2. Total Phenolic Content (TPC) Ethanolic Extract of *Aegle marmelos* Fruit Pulp

Parameter	Value
Regression equation	Y = 0.0073 x – 0.0017
Coefficient of determination (R^2)	0.9894
Sample absorbance (n=3)	0.0583 ± 0.0000
Total phenolic content (mg GAE/g extract)	8.22 ± 0.00

Note : data are presented as mean ± standard deviation (SD) (n=3)

The total phenolic content of the ethanolic extract of *Aegle marmelos* fruit pulp was determined to be 8.22 ± 0.00 mg GAE/g extract, indicating the presence of phenolic compounds at a moderate level. The high coefficient of determination (R^2 = 0.9894) confirms good linearity of the calibration curve and reliability of the analytical method. This value was calculated based on the regression equation y = 0.0073 x – 0.0017, as illustrated in Figure 1. Phenolic are well recognize for their ability to donate electrons or hydrogen atoms, thereby neutralizing free radicals and reducing oxidative damage (Chandrasekara et al., 2018).

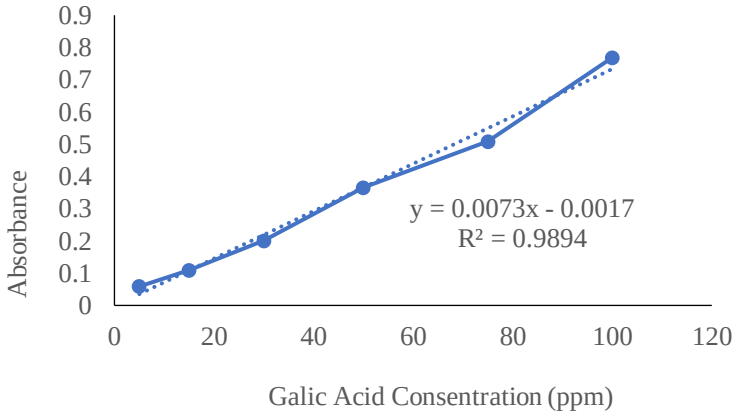


Figure 1. Gallic acid standard calibration curve used for total phenolic content (TPC) determination of the ethanolic extract of *Aegle marmelos* fruit pulp.

Although the toral phenolic content (TPC) value in this study is not particularly high, phenolic compounds may still contribute to biological activity, specially when acting in combination with other phytochemicals. Variations in phenolic content may arise from differences in plant origin, extraction methods, and

processing conditions, which can significantly affect compound recovery (Patra et al., 2015; Dai & Mumper, 2010).

Table 3. Antioxidant Activity of Ethanolic Extract of *Aegle marmelos* Fruit Pulp (DPPH Method)

Concentration (ppm)	% Inhibition (Rep 1)	% Inhibition (Rep 2)	% Inhibition (Rep 3)	Mean ± SD (%)
62.5	23.16	23.24	23.24	23.21 ± 0.05
125	29.59	26.59	26.59	26.59 ± 0.00
250	27.58	27.66	27.66	27.63 ± 0.05
500	29.79	29.79	33.29	30.96 ± 2.02
1000	67.57	67.57	67.50	67.52 ± 0.04

IC_{50} (ppm) : 712.18 ± 10.85
Noted : Data are presented as mean ± SD (n = 3)

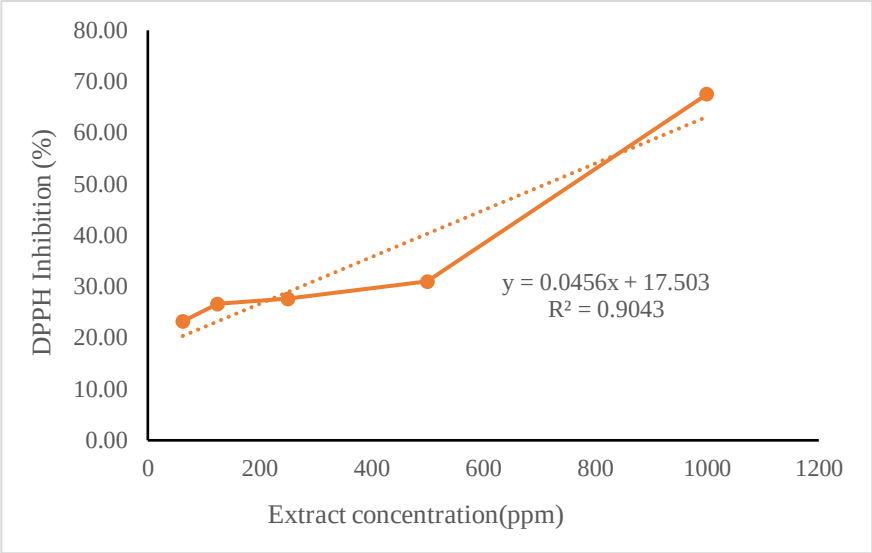


Figure 2. Relationship between extract concentration and percentage of DPPH radical inhibition of ethanolic extract of *Aegle marmelos* fruit pulp. The regression equation obtained was y = 0.0456 x + 17.503 with a coefficient of determination (R^2 = 0.09043), indicating a strong positive correlation between concentration and antioxidant activity

Table 4. Statistical Analysis of Antioxidant Activity

Test	Parameter	Value	Interpretation
Shapiro-Wilk	Normality	P < 0.05	Not normally distributed
Levene's Test	Homogenety	P < 0.001	Not homogeneous
Kruskal- Wallis	H (df = 4)	13.70	Significant
	P=value	0.008	Significant difference
Dunn Post Hoc (Holm)	62.5 vs 1000 ppm	P = 0.009	Significant
	Others	P > 0.05	Not significsnt

The antioxidant activity of the ethanolic extract of *Aegle marmelos* fruit pulp was evaluated using the DPPH radical scavenging assay, as presented in Table 3. The result demonstrated a concentration dependent increase in percentage inhibition, indicating that higher extract concentrations enhanced the ability to neutralize free

radicals. At the highest concentration (1000 ppm), the extract exhibited the strongest activity with an inhibition value 67.52 ± 0.04%.

Linier regression analysis revealed a positive correlation between extract concentration and antioxidant activity, with the equation y = 0.0456X + 17.503 and a coefficient of determination (R^2 = 0.9043), suggesting a strong linier relationship. This indicates that approximately 90% of the variation in antioxidant activity can be explained by the extract concentration, confirming a dose-dependent effect.

The IC_{50} value of the extract was calculated to be 712.18 ± 10.85 ppm, indicating relatively weak antioxidant activity according to commonly accepted classifications, where IC_{50} values above 500 ppm are categorized as low antioxidant potency. This result is consistent with the moderate total phenolic content (TPC) observed in this study, suggesting that the antioxidant capacity of the extract is influenced by the concentration of phenolic compounds.

From a statistical perspective, the data did not meet parametric assumptions, as indicated by Shapiro-Wilk and Levene's test ($p < 0.05$). therefore, the Kruskal-Wallis test was applied, revealing a significant difference among concentration groups ($p = 0.008$). However, post hoc analysis using Dunn's test with Holm correction showed that only the comparison between 62.5 and 1000 ppm remained statistically significant ($p = 0.009$), indicating that substantial differences in antioxidant activity occur primarily at widely separated concentrations.

The antioxidant activity in this study can be attributed to the presence of phenolic and flavonoid compounds, which act as hydrogen or electron donors to stabilize free radicals such as DPPH. These compounds are known to interrupt radical chain reactions and reduce oxidative stress. Which plays a critical role in cancer development and progression (Chandraseka et al., 2018; Dai and Mumper, 2010).

Although the antioxidant activity of the fruit pulp extract is relatively low compared to highly potent natural antioxidants, its biological relevance should not be underestimated. Phenolic compounds often act synergistically with other phytochemicals, contributing to overall bioactivity beyond what is reflected by IC_{50} values alone (León-González et al., 2015). This is particularly important in the context of anticancer research, where moderate antioxidant activity may still contribute to chemo preventive mechanisms through modulation of oxidative stress pathway.

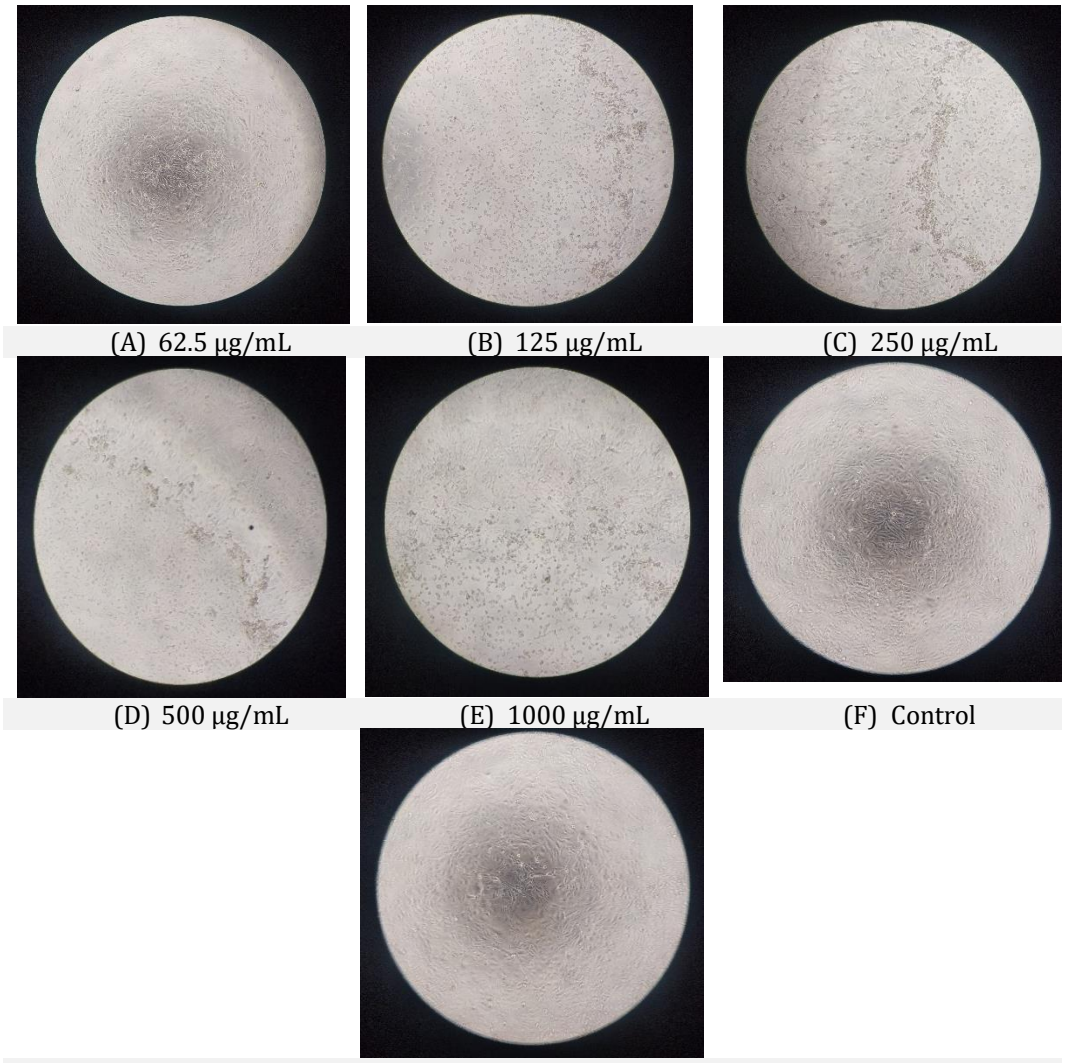
Recent studies have also emphasized that antioxidant activity measured by DPPH does not fully represent in vivo activity, as bioavailability and cellular interactions play critical roles (Shahidi and Ambigaipalan, 2015; Suleria et al., 2020). Therefore, the observed antioxidant potential of *Aegle marmelos* fruit pulp extract may still contribute to its biological effects, particularly when considered alongside its phytochemical composition and potential cytotoxic activity.

Table 5. Cell Viability of SKOV-3 Cells After Treatment with Ethanolic Extract of *Aegle marmelos* Fruit Pulp.

Concentration (µg/mL)	% Viability			Mean ± SD (%)
	Replication 1	Replication 2	Replication 3	
0	100.00	100.00	99.59	99.86 ± 0.24
31.3	99.27	105.99	100.20	101.82 ± 3.55
62.5	98.62	104.65	100.69	101.32 ± 3.09
125	96.30	101.64	100.30	99.41 ± 2.79
250	98.48	104.40	100.23	101.04 ± 3.02
500	103.40	103.66	100.00	102.35 ± 2.02

1000	99.53	99.60	100.37	99.83 ± 0.46

Note : Data are presented as mean ± SD (n = 3 independent experiments)



Noted : (A- E) SKOV-3 cells treted with increasing concentrtions (62.5 – 1000 µg/mL)
(F) untreated control
(G) DMSO control

Figure 3. Morphological appearance of SKOV-3 ovarian cancer cells treated with ethanolic extract of *Aegle marmelos* fruit pulp at different concentrations (62.5, 125, 250, 500, and 1000 µg/mL, along with untreated control and DMSO control, observed under an inverted microscope during the MTT assay. Untreated control cells exhibited normal morphology characterized by elongated shape and strong cell adhesion. Cells treated with lower concentration (62.5 – 125 µg/mL) showed minimal morphological changes and maintained

normal structure. At intermediate concentrations (250-500 µg/mL), cells began to exhibit morphological alterations, including cell shrinkage, rounding, and reduce adhesion. At the highest concentration (1000 µg/mL), more pronounced changes were observed, such as partial detachment and presence of cellular debris. The DMSO control group showed morphology comparable to untreated cells, indicating no significant solvent-induce effects.

Table 6. Summary of Statistical Analysis of SKOV-3 Cell Viability Following Treatment with Ethanolic Extract of *Aegle marmelos*.

Test	Result	Interpretation
Shapiro- Wilk	$p < 0.05$	Not normally distributed
Levene's test	$p < 0.001$	Variance not homogeneous
Kruskal-Wallis	$p < 0.001$	Significant difference between groups
Dunn post hoc (Holm)	Significant vs cisplatin only	Extract groups not significantly different

The cytotoxic potential of the ethanolic extract of *Aegle marmelos* fruit pulp against SKOV-3 ovarian cancer cells was evaluated using the MTT assay, with results expressed as percentage cell viability relative to untreated controls (Table 5). Across the tested concentration range (31.3 – 1000 µg/mL), the extract demonstrated minimal impact on cell viability, with values remaining close to or slightly above 100%, indicating the absence of a clear cytotoxic effect.

No dose dependent reduction in viability was observed. Instead, minor fluctuations occurred, including values exceeding 100%, which are commonly associated with increased mitochondrial metabolic activity rather than actual cell proliferation. This phenomenon reflects a known limitation of the MTT assay, where metabolic stimulation induced by phytochemicals can lead to overestimation of viable cells (Stockert et al., 2018).

Statistical analysis using the Kruskal-Wallis test revealed a significant overall difference among groups ($p < 0.001$) (Table 6). However, post hoc Dunn analysis (Holm correction) indicated that this significance was primarily driven by comparisons involving the positive control (Cisplatin), while no statistically meaningful differences were detected among extract-treated groups. This findings confirm that the extract does not exert significant cytotoxic activity against SKOV-3 cells under the experimental conditions.

Interestingly, despite the lack of cytotoxicity, morphological observations (Figure 3) revealed concentration dependent cellular alterations, particularly at higher doses. These included cell shrinkage, rounding, reduce adhesion, and partial detachment. Such changes are indicative of cellular stress responses and may represent early-stage apoptotic or cytoskeletal alterations. However, these morphological features were not accompanied by a corresponding decrease in viability, suggesting that the cells remained metabolically active.

This apparent discrepancy can be explained by several factors. First, the MTT assay measures mitochondrial activity rather than direct cell death, allowing cells undergoing early or reversible stress to maintain measurable viability. Second,

,morphological changes such as cell shrinkage and rounding may represent early apoptotic signaling that has not progressed to irreversible stages involving membrane disruption or loss of metabolic function (Elmore, 2007). Third, the bioactive compounds present in the extract particularly phenolics and flavonoids may exert cytostatic rather than cytotoxic effects, inducing temporary growth inhibition or cellular adaption without triggering cell death (Dai & Mumper, 2010).

The relatively moderate total phenolic content and weak antioxidant activity observed in this study may contribute to the limited biological potency of the extract. The concentration of active compounds may be insufficient to induce oxidative stress beyond the threshold required to active apoptosis or necrosis pathways (Candrasekara et al., 2018). Furthermore, SKOV-3 cells are known to posses strong adaptive and survival mechanisms, including enhances antioxidant defenses and anti-apoptotic signaling. Which may allow them to withstand sublethal stress conditions (Hanahan & Weinberg, 2011).

From a pharmacological perspective, these results suggest that the ethanolic extract of *Aegle marmelos* fruit pulp does not exhibit direct cytotoxic activity against SKOV-3 cells as a single agent. The observed morphological changes (Figure 3) indicate underlying biological activity at the cellular level. This implies that the extract may act through indirect mechanisms, such as modulation of cellular stress responses or interference with cell cycle regulation. Therefore, while the extract shows limited potential as a standalone cytotoxic agent, it may still hold relevance as a complementary therapeutic candidate. Further studies are warranted to explore its role in combinations that may enhance its anticancer efficacy (Wahyuni et al., 2025)

Conclusion

The ethanolic extract of *Aegle marmelos* fruit pulp contains phenolic compounds and exhibits moderate antioxidant activity, indicating the presence of bioactive constituents. However, it showed no significant cytotoxic effect against SKOV-3 cells, as reflected by consistently high viability and the absence of a dose dependent response. Morphological changes at higher concentrations suggest induction of sublethal cellular stress rather than cell death. These findings indicate that its anticancer potential is not mediated through direct cytotoxicity but may involve modulation of cellular processes. Further studies are needed to investigate synergistic effects and identify active compounds.

Declaration of Competing Interest

The authors declare that they have no competing interests

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