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Phytochemical analysis of Peganum harmala and Camellia sinensis seed extracts and evaluation of their cytotoxic effects on A431 (skin) cancer cell line

Summer Hussein Hillal[1] Azhar Hamza Hassan[2]
[1,2]**Department of Biology, College of Science, University of Babylon, Iraq**
[1]samar.kazem113@student.uobabylon.edu.iq.

Abstract:
Background: Cancer constitutes a significant public health crisis for humanity. Conventional cancer therapies often encompass chemotherapy or a mix of radiotherapy and chemotherapy. However, the harmful effects of synthetic drugs led to the search for new treatment options that are more compatible with the body. Bioactive chemicals derived from natural resources have transformed the field of medicinal chemistry. Consequently, these natural therapeutic substances have become crucial for the formulation of multifaceted treatment regimens in cancer therapy.

Objectives: The aims of extract, characterize, and evaluate the cytotoxic effects of *Peganum harmala* and *Camellia sinensis* seed extracts on both normal and cancer cell lines.

Methods: Quantitative analysis was employed to identify the phenolic components in Peganum harmala and Camellia sinensis seed extract via high-performance liquid chromatography. The cytotoxicity assay was conducted using the skin cancer cell line (A431) and the normal cell line (HEK293), all cultured in 96-well microtiter plates. was exposed to various dilution doses (100-3.12 µg/ml) of Peganum harmala and Camellia sinensis seed extract. The crystal violet assay was employed to evaluate cell viability.

Results: HPLC analysis indicated the presence of several anticancer compounds. Cytotoxicity results ($P \leq 0.005$) show that *Peganum harmala* extract greatly reduced the survival of A431 skin cancer cells at all tested amounts, while Camellia sinensis (green tea) extract only reduced survival in A431 skin cancer cells at higher amounts (100, 50, and 25 µg/ml). For the normal HEK293 cell line, *Peganum harmala* extract significantly reduced cell survival at lower amounts (3.125, 6.25, and 12.5 µg/ml), while *Camellia sinensis* extract only affected the cells at higher amounts.
Conclusion: The seed extracts of both plants had cytotoxic effects on cancer cell lines, with P. harmala demonstrating greater potency but also increased toxicity to normal cells. These findings indicate their potential as natural anticancer agents. In addition, in vivo studies are required to validate efficacy and evaluate safety.

Keywords: *Peganum harmala, Camellia sinensis*, A431cell line, HEK293, HPLC.

I. Introduction: Medicinal plants are considered valuable natural resources that have long contributed to human health and disease management. Historically, both plants and their bioactive compounds have helped a pivotal part in traditional medicine. Numerous medicinal herbs and their phytochemicals have demonstrated the ability to inhibit cancer development and progression (1) *Peganum harmala* L. (Zygophyllaceae) is a plant indigenous to arid regions from the eastern Mediterranean to northern India and is extensively distributed in Iran. Although the ingestion of P. harmala seeds can activate the central nervous system (CNS),

induce paralysis, and prove toxic at elevated levels, the smoke from these seeds is employed as an antibacterial remedy in traditional medicine (2) The plant's bioactive substances are b-carboline alkaloids, which are primarily found in the roots and seeds and include harmine, harmaline, harmol, and harmalol. (3) We were additionally intrigued by several categories of secondary metabolites from the herb, including phenolic chemicals, which serve as important the role of anti-oxidants in the prevention of cardiovascular disease, cancer, and diabetes The human organism undergoes oxidation, leading to oxidative stress, which substantially contributes to cancer formation. Multiple scientific research suggest that antioxidants may impede or maybe prevent the development of cancer. Consequently, interest in natural antioxidants, particularly those sourced from plants, has markedly increased in recent times (4) Multiple studies have validated that green tea (*Camellia sinensis*) comprises bioactive compounds—including polyphenols, caffeine, L-theanine, and polysaccharides—that demonstrate considerable pharmacological effects. These components exhibit antioxidant, anticancer, and neuroprotective properties. Epigallocatechin gallate (EGCG), a principal catechin in camellia sinensis has exhibited the capacity to diminish oxidative stress and impede the development of cancer cells. L-theanine also enhances neuroprotection by regulating neurotransmitter activity and facilitating relaxation without inducing drowsiness (5) Studies have shown that the strong antioxidant properties of green tea are attributed to catechins of EGCG and EGC The three adjacent hydroxyl groups on the B-ring of EGCG, GCG, EGC, and GC are more effective in scavenging free radicals than the two adjacent OH groups of ECG, CG, and EC (6)

II. Materials and Methods

Plant collection

Peganum harmala and *Camellia sinensis* (green tea) seeds brought from local market in Iraq. The plants had been identified by Dr. Neddaa Adnan from the University of Babylon's Plant Herbarium, Department of Biology, College of Science.

Preparation of plant extract

Ultrasonic The extraction of phenolic chemicals. Phenolic compositions were extracted from homogenized plant material. To 20 g of the pulverized plant, 100 ml of chloroform was introduced, and the combination was treated to an electric vibrator for 3 hours to extract fat from the sample. Thereafter, the chloroform layer was extracted, and the sample was subjected to heating at 50 °C to guarantee the total evaporation of chloroform remnants. Ultimately, 10 g of the desiccated sample was employed for extraction utilizing a 70/30 ethanol/water solventThe extraction procedure was conducted with an ultrasonic bath (USA) at ambient temperature for one hour. After filtration, 5 mL of the liquid extract was employed to determine the extraction yield. We eliminated the solvent using a rotary evaporator under vacuum conditions in Slovenia, followed by drying at 40°C until we achieved a consistent mass. Dry extracts were preserved in glass bottles at 4°C to avert oxidative degradation before analysis.

The measurement of certain phenolic compounds was conducted via reversed-phase HPLC analysis, utilizing a SYKAM HPLC chromatographic system fitted with a UV detector and a -C18-OSD column (25 cm, 4.6 mm). The column temperature was 30 degrees Celsius. The gradient elution technique was executed utilizing eluent A (methanol) and eluent B (1% formic acid in water (v/v)), as detailed below: Initially, we used 40% B from 0 to 4 minutes and 50% B

from 5 to 15 minutes, maintaining a flow rate of 0.7 mL/min. The injected volume of samples and standards was 100 µL, executed automatically by an autosampler. The spectra were obtained at 280 nm.

Cell line

Cancerous cell lines A431, and the normal cell line HEK293 were grown in RPMI-1640 media with 10% fetal bovine serum (FBS) to supply the nutrients necessary for their growth. We incubated the cells at 37°C in a humidified environment with 5% CO_2.

Cytotoxicity assay

The crystal violet assay was employed to assess cytotoxicity. The HEK293 normal cell line was seeded onto 96-well plates at a density of 5×10^5 cells per well. Cells were treated with various concentrations (100, 50, 25, 12.5, 6.25, and 3.12 µg/ml) of the aqueous extract, with three replicates for each concentration. Six wells functioned as untreated controls. In the same way, A431 skin cancer cells were placed in 96-well plates and given the same amounts (100–3.12 µg/ml) of the water extract. The plates were sealed with self-adhesive plastic lids and incubated at 37°C for 48 hours. Subsequent to incubation, the culture media was discarded, and the wells were rinsed with 200 µL of sterile PBS. Thereafter, 50 µL of crystal violet dye was introduced to each well, and the plates were incubated for an additional 20 minutes. Following staining, the supernatant was eliminated, the wells were rinsed with distilled water, and the plates were allowed to dry at ambient temperature. Cell viability was determined as a percentage in relation to the untreated control cells (8)

statistical analysis

Statistical significance of the differences in the data between the experimental group and the control group, a two-way analysis and Tukey's of variance was carried out using the SPSS 25 computer program. The statistical significance was determined by evaluating the p-value ($p \leq 0.0001$, $p \leq 0.005$).

Ethical Approval

This research did not involve any humans or animals to obtain their consent. According to the document with the number B241002 and the date October 27, 2024, the study procedures, subject information, and agreement form were evaluated and approved by a local ethics committee of the University of Babylon, College of Science's Biology Department.

III. Result

Phenolic content of both *Peganum harmala* and *camellia sinensis* extract according to HPLC (High Performance Liquid Chromatography analysis as following:

Table1**:** concentrations of phenolic compounds in both *Peganum harmala* and *Camellia sinensis* seeds extract

Name	*Camellia sinensis* (ppm)	*Peganum harmala* (ppm)
Caffeic acid	177.9	49.8
Chlorogenic acid	114.1	55.7

Ellagic acid	88.4	51.0
Epicatechin	71.3	132.5
Gallic acid	184.9	162.0
Hydrobenzoic acid	41.6	70.5
Rutin	125.3	70.1
	68.0	90.7
	154.6	68.9

Table 2**:** Phytochemicals identified in the *Peganum harmala seeds* extract by HPLC

No							
1							
					12.00	12.00	
					10.00	10.00	
					11.00	11.00	
					14.00	14.00	
					8.00	8.00	
					11.00	11.00	
					9.00	9.00	
					10.00	10.00	
					100.00	100.00	

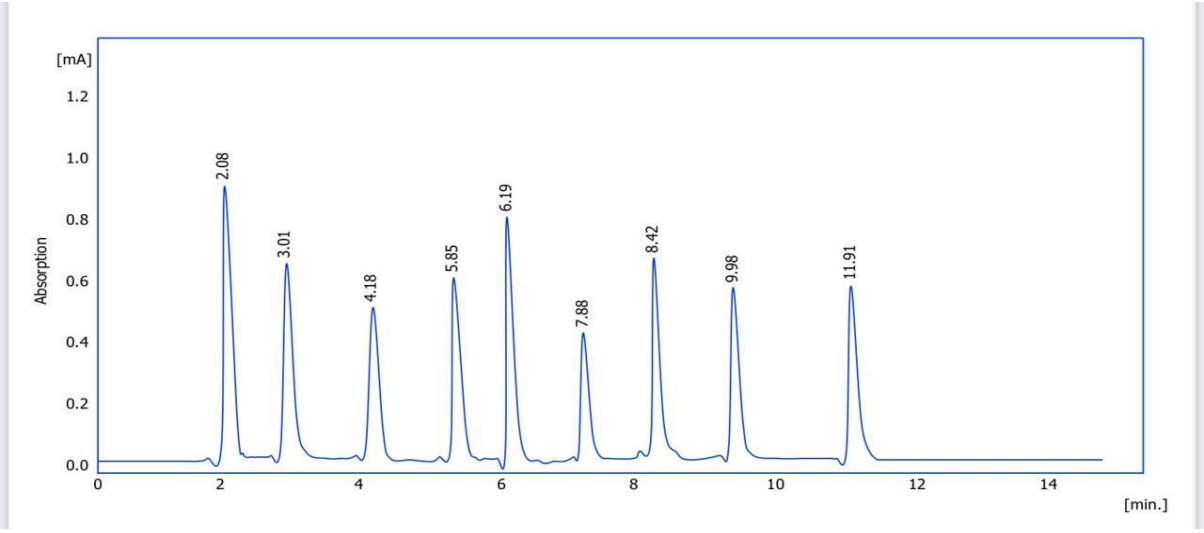

Figure (1 HPLC chromatogram of *Peganum harmala* seed extract.

Table 3: Phytochemicals identified in the *Camellia sinensis seeds* extract by HPLC

No							
1							
					8.00	8.00	
					11.00	11.00	
					13.00	13.00	
					11.00	11.00	
					11.00	11.00	
					8.00	8.00	
					10.00	10.00	
					12.00	12.00	
					100.00	100.00	

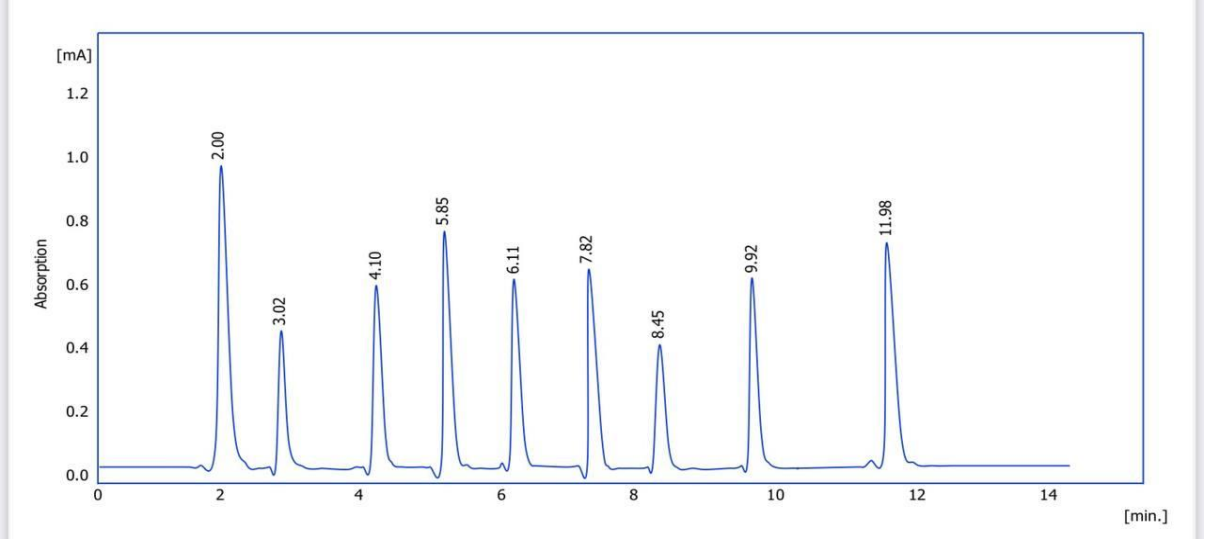

Figure (2 HPLC chromatogram of *Camellia sinensis* seeds extract.

The study examined the cytotoxicity effect of an extract of *Peganum harmala* seed on an A431 skin cancer cell line at different concentrations after incubation for 48 hours.

We applied different amounts (3.12-100 µg/ml) of *Peganum harmala* seed extract to the skin cancer A431 cell line. Cells were incubated for 48 hours at 37°C, and then viability was

assessed using the crystal violet test.

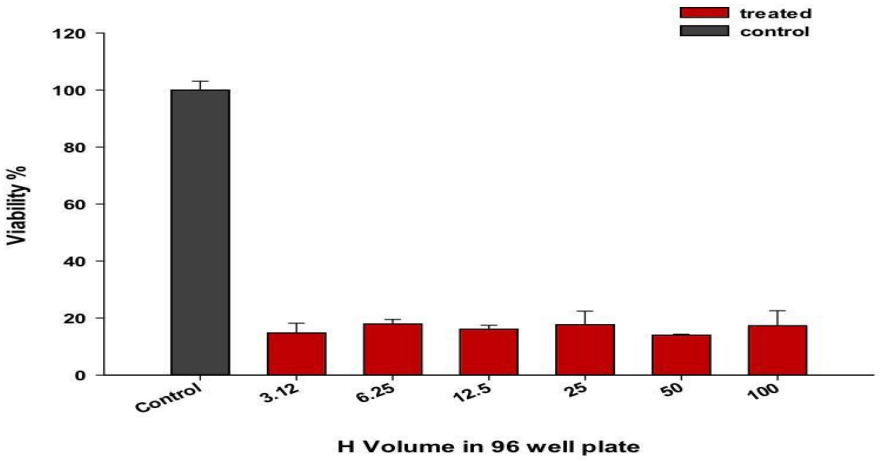

Figure (3 cells viability percentage of A431 cell line at different concentrations of *Peganum harmala* extract after incubation for 48 hours by crystal violet assay

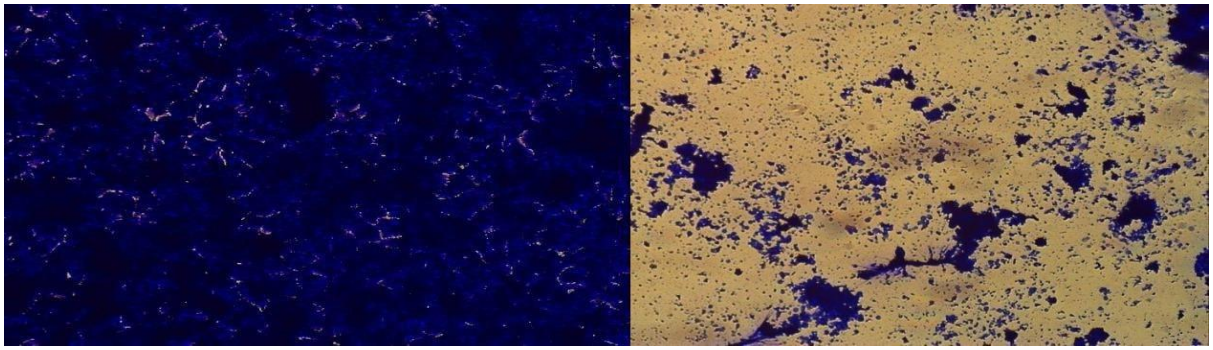

Figure (4 The A431 cell line under microscope after treated with serial concentrations (3.12-100 µg/ml) of *peganum Harmala* extract and Untreated A431 skin cancer cells (Control group).

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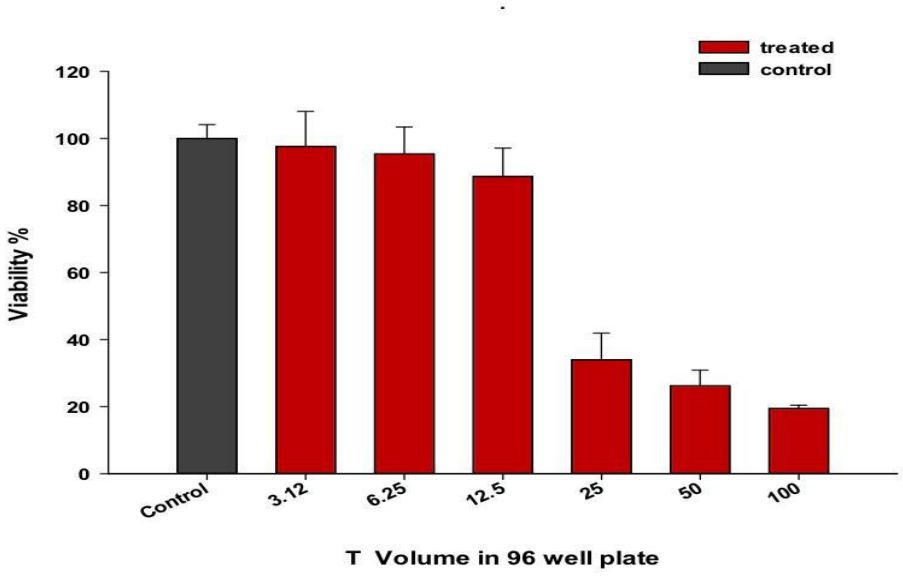

Figure (5 cells viability percentage of A431 cell line at different concentrations of *Camellia sinensis* extract after incubation for 48 hours by crystal violet assay.

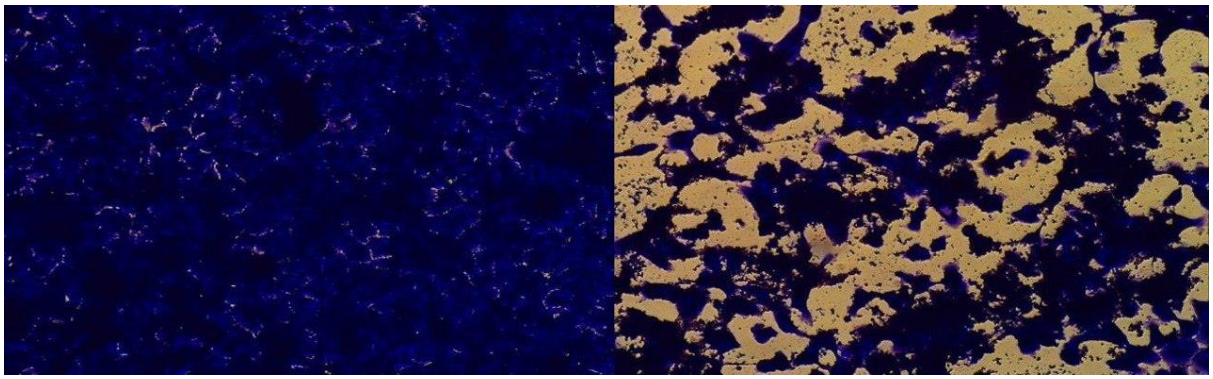

Figure (6) The A431 cell line under microscope after treated with serial concentrations (3.12-100 µg/ml) of *C. sinensis* extract and Untreated A431 skin cancer cells (Control group).

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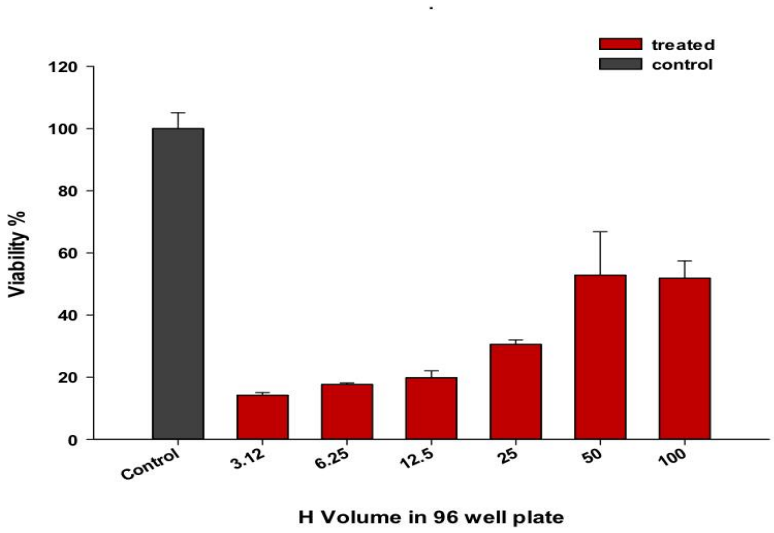
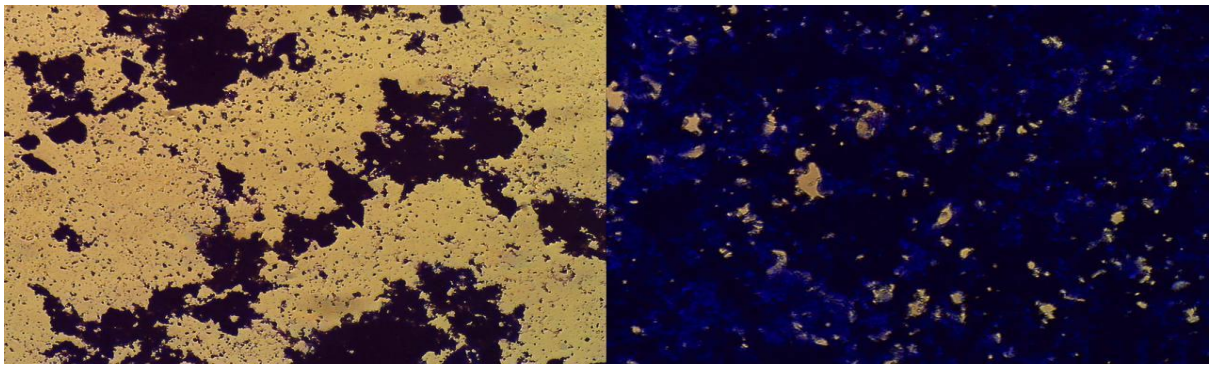


Figure 7 cells viability percentage of HEK293 cell line at different concentrations of *Peganum harmala* extract after incubation for 48 hours by crystal violet assay.



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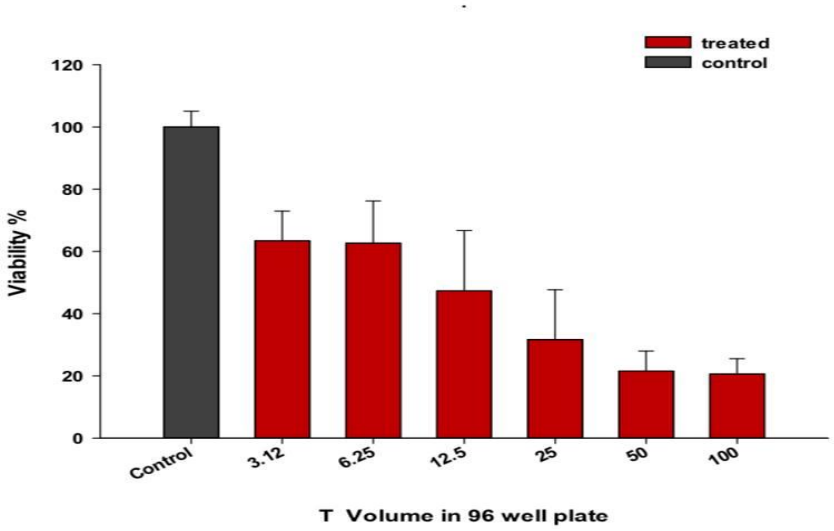
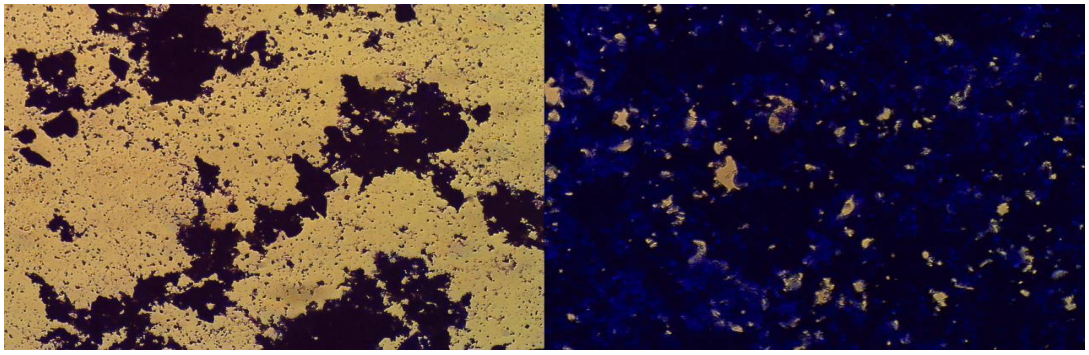


Figure 9) cells viability percentage of HEK293 cell line at different concentrations of *Camellia sinensis* extract after incubation for 48 hours by crystal violet assay.



Discussion

Numerous therapeutic plants possess anticancer effects, and the use of medicinal plants for cancer prevention or treatment has been done for many years. Plant-based medicine is gaining popularity as individuals increasingly embrace organic and simplistic lifestyles. Global research on plants has been conducted to explore novel and potential sources of anticancer agents, while synthetic anticancer drugs have considerable limits due to adverse effects and drug interactions (9) The phytochemical test of the plant extract used in this study verified the presence of flavonoids and phenolic compounds. Because of these components, this species may have some medicinal promise. Our findings are consistent with earlier research that used the following extraction solvents: water, ethanol, petroleum ether, chloroform, and ethyl acetate. The following components were evaluated as follows: flavonoids (quercetin equivalent 0 to 3.12 g/kg), anthocyanins (cyanidin equivalent 0 to 20.56 mg/kg), tannins (catechin equivalent 0 to 25.27 g/kg), and phenolics (gallic acid equivalent 2.48 to 72.52 g/kg)(10)

The cytotoxic effects of *peganum harmala* and *camellia sinensis* seed extract on the normal HEK293 cell line, and A431 skin cancer cells were assessed to build a theoretical basis for their usage in cancer treatment.

The extract of Peganum harmala seeds demonstrated a significant ($P \leq 0.005$) reduction in the viability of the HEK293 cell line relative to the control group solely at the lowest concentrations (3.125, 6.25, and 12.5 μg/ml). On the other hand, the extract of Camellia sinensis showed a notable drop in the survival of the HEK293 cell line compared to the control group at higher amounts (100, 50, and 25 μg/ml). The findings from the extract in figure (7) are consistent with the research on the cytotoxicity of Peganum harmala on the HEK293 cell line, which demonstrated the extract of harmala seeds exhibited significant cytotoxicity to HEK293 cells at concentrations exceeding 0.5 mg/mL, resulting in over 50% cell mortality (11)

A431 (ATCC-CRC 1555) is a human cancer cell line derived from an epidermoid carcinoma of the vulva in an 85-year-old female patient. Researchers used this cell line to study how skin cancer develops and to test the harmful effects and growth-inhibiting properties of natural and man-made substances (12) The extract of Peganum harmala significantly reduced A431 cell viability over all concentrations, as seen by the results in Figure 4. Comparable results were observed with HCT116 colon cancer cells and MDA-MB-231 breast cancer cells (13)

The observations indicate that the anticancer properties of P. harmala may be consistent across several cell types. The present study is the first report of such effects on A431 cells, suggesting a potential new application for P. harmala in skin cancer research. Camellia sinensis exhibits a notable decrease in A431 cell viability solely at high concentrations (100, 50, and 25 μg/ml). The results match the study by Singh and Katiyar (2013), which found that EGCG, a key ingredient in green tea, can kill A431 skin cancer cells by stopping β-catenin signaling, leading to fewer living cells and more cell death. This finding validates the possible anti-cancer capabilities of Camellia sinensis extract (14)

Conclusion

The seed extracts of both plants had cytotoxic effects on cancer cell lines, with P. harmala showing higher potency alongside heightened toxicity to normal cells. These findings suggest their potential as natural anticancer agents. Furthermore, in live experiments are necessary to confirm efficacy and assess safety.

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