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Novel Serum-based Approach for Evaluating Phytochemical Polyphenol Ellagic Acid: Antioxidant and Cytotoxic Assessment by DPPH and MTT Assays

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ABSTRACT

Background: Ellagic acid, a phytochemical polyphenol found in fruits such as *Fragaria*, *Vitis vinifera* and *Punica granatum*, exhibits potent antioxidant and anticancer activities. Its therapeutic application is limited by poor solubility and low bioavailability. To overcome these limitations, a novel serum-based polymeric nanoparticle formulation was developed to enhance topical delivery and improve biological efficacy.

Methods: The antioxidant activity of ellagic acid nanoformulation was evaluated using the DPPH assay, where serial dilutions were incubated with DPPH solution and absorbance was measured at 517 nm to calculate radical scavenging activity (RSA) and IC_{50} values. Cytotoxicity was assessed in michigan cancer foundation-7 (MCF-7) breast cancer cells using the MTT assay, where cells were treated with the nanoformulation at concentrations of 20-100 µmol/L for 48 hours and cell viability and IC_{50} values were determined.

Result: The ellagic acid nanoformulation exhibited concentration-dependent DPPH radical scavenging activity ranging from 24.28% to 54.63%, with an IC_{50} of ~50 µg/mL, compared to ascorbic acid (46.77-78.90%; IC_{50} ~12.5 µg/mL). In cytotoxicity studies, the formulation inhibited MCF-7 cell proliferation from 14.09% at 20 µmol/L to 92.49% at 100 µmol/L, with an IC_{50} of 54.88 µmol/L, whereas paclitaxel (0.1 µmol/L) produced 66.45% inhibition. These results confirm that the serum-based ellagic acid nanoformulation possesses significant antioxidant and anticancer potential, supporting its therapeutic applicability.

Key words: Antioxidant activity, Cytotoxicity, DPPH assay, Ellagic acid, MCF-7 cells, MTT assay, Polymeric nanoparticles, Serum formulation.

INTRODUCTION

Ellagic acid, a phytochemical polyphenol naturally abundant in fruits such as *Fragaria*, *Vitis vinifera* and *Punica granatum*, has gained considerable interest for its diverse pharmacological activities, particularly anticancer potential (Wang *et al*., 2017; Beshbishy *et al*., 2019). It exhibits effectiveness against multiple malignancies, including colon, prostate, esophageal, skin and breast cancers, through mechanisms such as apoptosis induction, inhibition of abnormal proliferation, antioxidant activity and modulation of key molecular signaling pathways (Boehning *et al*., 2018; Golmei *et al*., 2024). In breast cancer models, ellagic acid suppresses growth in both estrogen-responsive (MCF-7) and estrogen-unresponsive (MDA-MB-231) cell lines, partly by inhibiting angiogenesis *via* VEGFR-2 tyrosine kinase signaling (Hussain *et al*., 2020; Cristy *et al*., 2024; Yap *et al*., 2021).

Beyond its anticancer effects, ellagic acid is a potent antioxidant, capable of scavenging reactive species such as hydroxyl, peroxyl, NO, and peroxynitrite (Sabando *et al*., 2020; Milani *et al*., 2018). Its chemical structure, featuring two lactone rings and four hydroxyl groups, facilitates electron donation to neutralize free radicals and enhances enzymatic antioxidant defenses including superoxide dismutase and catalase (Pandey and Bhatt, 2016; Deepika *et al*., 2023; Hosseinzadeh *et al*., 2021). Additionally, ellagic acid chelates metal ions, preventing Fenton-type radical generation and its derivatives, such as urolithins, mitigate hepatic damage and inhibit cancer cell proliferation (Agrawal and Kulkarni, 2020; Chen *et al*., 2022).

Despite its potent bioactivities, clinical applications are limited by poor solubility and low bioavailability, necessitating advanced strategies like nanoencapsulation or complexation to improve therapeutic efficacy (Nyamba *et al*., 2020; Karumuru *et al*., 2025; Zuccari *et al*., 2020). Given its robust antioxidant, anti-inflammatory and anticancer properties, ellagic acid remains a promising natural compound for pharmaceutical and nutraceutical

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development (Živković *et al*., 2017; Ishola *et al*., 2024; García *et al*., 2019; Gupta *et al*., 2015; Golmei *et al*., 2024).

To overcome the poor solubility and limited bioavailability of ellagic acid, serum-based polymeric nanoparticles were developed for topical delivery to enhance its efficacy. Excipients were selected and placebo batches were evaluated for finalization of Excipient, with the final combination chosen based on particle size and stability. EA-loaded nanoparticles were then prepared and entrapment efficiency was determined. Characterization studies, including FTIR and XRD analyses, confirmed the retention of characteristic functional groups and crystalline peaks of EA, indicating successful encapsulation. The dried nanoparticles were incorporated into an oil-based serum formulation, which demonstrated suitable viscosity and drug content for topical application. In vitro studies confirmed enhanced solubility and diffusion of ellagic acid when formulated in serum formulation. These findings highlight that the EA-loaded serum nanoparticles provide a safe, stable and effective platform for antioxidant delivery, addressing previous limitations in EA bioavailability and therapeutic potential.

This study evaluated the anticancer and antioxidant effects of a topical ellagic acid serum formulation. Cytotoxicity was assessed on MCF-7 breast cancer cells using the MTT assay, a standard colorimetric method that quantifies cell viability based on the reduction of MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) by mitochondrial enzymes into purple formazan, with color intensity proportional to the number of viable cells (Mirmalek *et al*., 2020; Das *et al*., 2021). The MCF-7 cell line, derived from human breast adenocarcinoma and expressing estrogen receptors, serves as a well-established in vitro model for breast cancer research. Antioxidant activity was evaluated using the DPPH assay, a rapid, simple and widely used spectrophotometric method for assessing free radical scavenging capacity (Assunção *et al*., 2017). The assay directly measures hydrogen-donating ability, making it suitable for high-throughput screening of extracts and pure compounds (Correddu *et al*., 2019; Zuccari *et al*., 2020). It is based on the reduction of the stable violet DPPH radical to its non-radical yellow form (DPPH-H), with a corresponding decrease in absorbance at 517 nm (Aladejana, 2023).

MATERIALS AND METHODS

Material

Standard laboratory reagents and material were used in this study 96-well microplates SPL Biosciences, DPPH (2,2-diphenyl-1-picrylhydrazyl) manufacture by Sisco Research Laboratories Pvt. Ltd., Alcohol purchased from Sigma Aldrich, Ascorbic Acid purchased from RV lifesciences Ltd and Becton Dickinso Medical (s) Pvt. Ltd. Syringe was used. Dulbecco's Modified Eagle's Medium (DMEM), Fetal Bovine Serum (FBS), Anti- solution and trypsin-EDTA manufacture by Gibgo USA, 96-well microplates SPL Biosciences and MCF-7 (Procured from NCCS Pune)cell line. The study was conducted in Pharmaceutical Department of Laddhad college of Pharmacy,Yelgaon Buldana Maharashtra and study conducted from February 2023 to June 2025.

DPPH assay

Reagent preparation

DPPH solution

To prepare the DPPH solution, 3.94 milligrams of DPPH were dissolved in 100 milliliters of ethanol and the freshly prepared solution was used for the antioxidant assay.

Standard/test sample stock solution

Stock solutions of ascorbic acid (reference) and the test Serum formulation were initially prepared at 1 mg/mL. These were further serially diluted to obtain concentrations of 10, 20, 40, 60, 80 and 100 µg/mL. This dilution range was employed to calculate the IC_{50} defined as the concentration required to inhibit 50% of radical activity.

Experimental procedure

For the antioxidant evaluation, 100 µL of DPPH solution was dispensed into each well, while 100 µL of methanol served as the blank. To these, 100 µL of varying concentrations

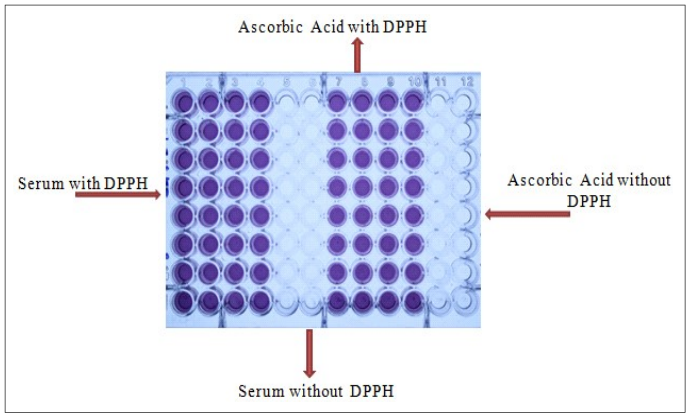

Fig 1: The absorbance of the samples in 96-well plates was measured after incubation to assess DPPH radical scavenging activity.

of ascorbic acid (standard) and the test sample (serum containing ellagic acid-loaded polymeric nanoparticles) were added (Fig 1). The plate was incubated in the dark at room temperature for 20 minutes. Absorbance was then recorded at 517 nm using a UV spectrophotometer. Radical scavenging activity (RSA) was determined using the formula: Radical scavenging activity (RSA) was calculated as:

$$\frac{\text{Control absorbance - Sample absorbance}}{\text{Control absorbance}} \times 100$$

This calculation provided a quantitative measure of the sample's ability to scavenge free radicals (Kumar *et al*., 2024) (Patil *et al*., 2025) (Beniwal *et al*., 2025).

MTT assay

Cell culture and treatment

MCF-7 breast cancer cells were maintained in high-glucose medium supplemented with 10% fetal bovine serum and 1% antibiotic-antimycotic solution. The cells were treated with the ellagic acid formulation at concentrations of 20-100 micromoles per liter to assess dose-dependent cytotoxic effects. Paclitaxel, a well-established anticancer drug, was included as a positive control at 100 nanomoles per liter (Wang *et al*., 2017).

MTT assay

Cells were treated with the ellagic acid serum formulation for 48 hours and cell viability was evaluated using the MTT assay, which measures the activity of mitochondrial enzymes in living cells. During the assay, metabolically active cells reduce the yellow tetrazolium salt to insoluble purple formazan crystals over a 4-hour incubation. The formazan product was quantified by measuring absorbance at 560 nm using an ELISA reader and Cell viability (%) was calculated as:

$$\frac{\text{Abs\_treated}}{\text{Abs\_untreated}} \times 100$$

Where

Abs_treated and Abs_untreated = The absorbance of treated and untreated cells, respectively.

This method provides a quantitative assessment of the cytotoxic effect of the ellagic acid formulation (Van *et al*., 2011) (Singh *et al*., 2024).

Preparation of Ellagic acid formulation

To prepare a 100 µmol/L solution, 303.0 µL of the test serum formulation was mixed with 9.697 mL of distilled water. Lower concentrations were prepared by serial dilution.

Results and Discussion

Result

The antioxidant activity of topical ellagic acid serum was assessed in comparison with ascorbic acid. Both exhibited concentration-dependent scavenging, with ellagic acid nanoformulation showing 24.28%-54.63% activity and ascorbic acid 46.77%-78.90% (Fig 2). The IC_{50} values were about 50 µg/mL for the serum and 12.5 µg/mL for ascorbic acid, confirming both possess antioxidant potential, though with differing potencies.

MTT assay

The results show that the topical serum formulation of ellagic acid exhibited a concentration-dependent cytotoxic effect on MCF-7 cells (Fig 3). The % cell inhibition increased with increasing concentrations of the nanoformulation, ranging from 14.09% at 20 µmol/L to 92.49% at 100 µmol/L. The positive control, paclitaxel (0.1 µmol/L), showed a % cell inhibition of 66.45%, indicating significant cytotoxic activity. Notably, the IC_{50} value of paclitaxel was not calculated in this study due to the single concentration used, but its potent cytotoxic effect at a low concentration (0.1 µmol/L) highlights its efficacy. In contrast, the serum formulation of ellagic acid demonstrated potent cytotoxic activity against MCF-7 cells, with an IC_{50} value of 54.88 µmol/L (Table 1). The IC_{50} study is crucial in determining the efficacy of a compound, as it represents the concentration required to inhibit 50% of cell growth. A lower IC_{50} value indicates higher potency, making it an essential parameter in evaluating the potential of anticancer agents. These results suggest that the nanoformulation of ellagic acid warrants further investigation for its potential anticancer properties.

The findings of this study demonstrate the potential of topical ellagic acid serum nanoformulation as an antioxidant and anticancer agent. The serum exhibited concentration-dependent antioxidant activity, with ellagic acid nanoformulation showing significant radical scavenging potential (24.28%-54.63% activity) and an IC_{50}

Table 1: Percentage inhibition of MCF-7 cells following treatment with different concentrations of ellagic acid serum.

Sample ID	Concentration of sample (µmol/lit)	Average per cent viability	% Inhibition	IC_{50} value of ellagic acid serum
Control	0	100.0000	0.0000	
Paclitaxel	0.1	33.5478	66.4522	
E.A serum	20	85.9142	14.0858	54.88 µmol/lit
	40	67.4641	32.5359	
	60	40.7832	59.2168	
	80	22.5256	77.4744	
	100	7.5148	92.4852	

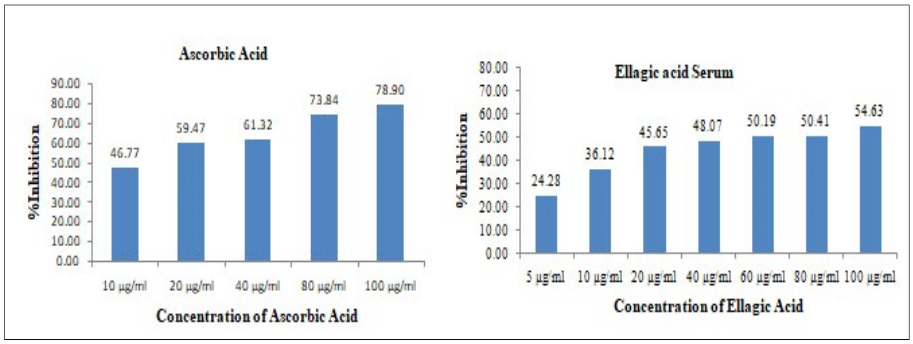

Fig 2: DPPH scavenging ability percentage of standards and serum.

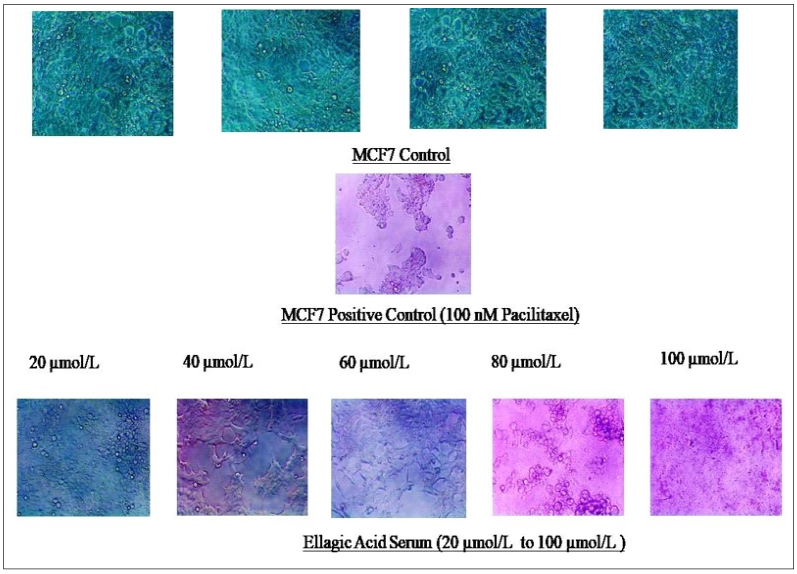

Fig 3: Representative images showing the viability of MCF-7 cells after treatment with varying concentrations of ellagic acid serum.

value of approximately 50 µg/mL. In comparison, ascorbic acid, a well-known antioxidant, showed higher antioxidant activity (46.77%-78.90% activity) and a lower IC_{50} value of 12.5 µg/mL. These results indicate that while ascorbic acid has stronger antioxidant capacity, the ellagic acid nanoformulation still possesses notable antioxidant potential.

The study also evaluated the cytotoxic activity of the ellagic acid nanoformulation against MCF-7 breast cancer cells. The results show a concentration-dependent cytotoxic effect, with increasing concentrations of the nanoformulation leading to enhanced cell inhibition (14.09% at 20 µmol/L to 92.49% at 100 µmol/L). The IC_{50} value of 54.88 µmol/L indicates potent cytotoxic activity, suggesting that the nanoformulation has potential anticancer properties.

The use of nanoformulation technology has likely contributed to the enhanced bioavailability and efficacy of ellagic acid, addressing its limitations of poor solubility and stability. These findings are consistent with previous studies highlighting the antioxidant and anticancer potential of ellagic acid.

Overall, this study provides evidence for the potential benefits of topical ellagic acid serum nanoformulation in antioxidant and anticancer applications. Further research is warranted to explore its therapeutic potential, including *in vivo* studies and clinical trials, to fully harness its benefits in preventing and treating cancer and other oxidative stress-related disorders.

CONCLUSION

The developed topical serum-based polymeric nanoparticles of ellagic acid demonstrated enhanced antioxidant and anticancer potential, overcoming the limitations of poor solubility and bioavailability. The formulation exhibited effective DPPH radical scavenging and notable cytotoxicity against MCF-7 breast cancer cells, confirming the biological efficacy of ellagic acid in a topical delivery system. These findings validate ellagic acid as a potent plant-derived phytochemical and highlight the significance of medicinal plants as sustainable sources of functional compounds capable of mitigating oxidative stress.

Moreover, the topical serum formulation of ellagic acid holds promise for cosmeceutical applications, leveraging its antioxidant properties to protect the skin from environmental stressors and prevent premature aging. Its dual role as an antioxidant and anticancer agent makes it

an attractive ingredient for skincare products and therapeutic regimens. The potential use of this formulation in cosmeceuticals and pharmaceuticals could provide a safe, stable and effective solution for promoting skin health and preventing skin-related disorders.

Overall, this study provides evidence for the potential benefits of topical ellagic acid serum nanoformulation in antioxidant and anticancer applications. Further research is warranted to explore its therapeutic potential, including in vivo studies and clinical trials, to fully harness its benefits in preventing and treating cancer and other oxidative stress-related disorders.

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Disclaimers

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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