International Journal of Medical Science and Research
www.medicaljournals.in
Online ISSN: 2664-8768
Received: 05-01-2026, Accepted: 04-02-2026, Published: 19-02-2026
Volume 8, Issue 1, 2026, Page No. 35-44

Development and characterization of Grateloupia lithophila silver nanoparticles and their in-*vitro* anti-gastric cancer assessment compared to ethanolic extract of Grateloupia lithophila using AGS cell line

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Abstract
Marine red algae represent an invaluable source of structurally diverse secondary metabolites with significant pharmacological potential. The present study investigates the anti-gastric cancer efficacy of *Grateloupia lithophila* against human gastric adenocarcinoma (AGS) cell lines through the MTT assay, employing both ethanolic extract (EEGL) and a green-synthesized silver nanoparticle formulation derived from the aqueous extract (AgNPs-AEGL). The silver nanoparticles were prepared using an eco-friendly reduction process and characterized by UV-Visible spectroscopy, FT-IR and scanning electron microscopy (SEM), confirming nanoscale formation and surface stability. Comparative cytotoxic assessment revealed a concentration-dependent reduction in AGS cell viability, with IC_{50} values of 76.43 µg/mL for EEGL, 34.23 µg/mL for AgNPs-AEGL, and 25.93 µg/mL for the standard chemotherapeutic agent 5-fluorouracil (5-FU). The enhanced cytotoxic potential of AgNPs-AEGL is v cellular shrinkage, membrane blebbing, and nuclear condensation corroborated apoptotic cell death. These findings highlight the synergistic interplay between the phytochemical matrix of *G. lithophila* and nanostructured silver, emphasizing its promise as a potent marine-derived nanotherapeutic for gastric carcinoma management. The study establishes a strong pharmacological rationale for further mechanistic and *in vivo* evaluation of *G. lithophila*-based formulations in cancer therapeutics.

Keywords: *Grateloupia lithophila*, silver nanoparticles, gastric carcinoma, AGS cell line, MTT assay, cytotoxicity, 5-fluorouracil, nanotherapeutics

Introduction

Marine algae have emerged as prolific reservoirs of structurally diverse secondary metabolites with significant pharmacological potential. Among them, red algae (Rhodophyta) are particularly distinguished for their unique bioactive constituents, including sulfated polysaccharides, flavonoids, terpenoids, and fatty acids, which exhibit potent antioxidant, anti-inflammatory, and cytotoxic properties [3, 13]. The exploration of marine algal metabolites has thus gained momentum as a promising strategy in natural product-based drug discovery, particularly for the development of novel anticancer agents with minimal systemic toxicity [5, 11].

Grateloupia lithophila Borgesen (Halymeniaceae), a red marine alga, has recently attracted attention for its diverse phytochemical composition and associated therapeutic prospects [45, 22, 9, 10, 33]. Despite growing evidence of its biological activity, the pharmacological potential of *G. lithophila*, particularly against gastric malignancies, remains largely unexplored. Gastric carcinoma continues to represent a major global health burden, characterized by high mortality rates and limited therapeutic efficacy of conventional chemotherapeutics such as 5-fluorouracil (5-FU), which often induce adverse effects and drug resistance [4, 8].

To overcome these challenges, nanotechnology-based approaches have emerged as a transformative tool in enhancing the delivery, bioavailability, and therapeutic index of natural compounds [18]. In this context, the green synthesis of silver nanoparticles (AgNPs) using algal extracts offers a sustainable and biocompatible strategy for developing targeted anticancer formulations [19, 1]. The nanoscale properties of AgNPs not only facilitate efficient cellular penetration but also augment oxidative stress-mediated apoptosis in tumor cells [17, 7].

The present study focuses on the formulation and evaluation of silver nanoparticles synthesized from the aqueous extract of *Grateloupia lithophila* (AgNPs-AEGL) and compares their cytotoxic efficacy with the ethanolic extract (EEGL) and the standard chemotherapeutic agent 5-fluorouracil. The *in vitro* anticancer potential was assessed using the MTT assay on AGS human gastric adenocarcinoma cell lines. This investigation aims to elucidate the synergistic role of phytochemical-rich algal extracts and nanotechnology in enhancing anticancer efficacy, thereby establishing *G. lithophila* as a promising candidate for future marine-based nanotherapeutic development against gastric cancer.

Materials and methods

Collection and authetication of algae material: The Red algae of *Grateloupia lithophila Borgesen* were collected from, Pudhumadam in Ramanathapuram district in Tamil Nadu, India during the month of March 2025. The algal samples were carefully rinsed with seawater followed by freshwater to remove epiphytes and sand particles. Samples were shade dried and stored in airtight containers. The plant was authenticated by marine scientist *Dr.veeraguranathan,* chief scientist of CSIR-CMCRI, Mandapam Camp, Ramanathapuram district and confirmed as *Grateloupia lithophila Borgesen* (Rhodophyta: Halymeniaceae).

Extraction of algae materials: The coarsely powdered *Grateloupia lithophila* was subjected to extraction with ethanol in the proportion of 1: 4 and the resultant residue, designated as the ethanolic extract of *Grateloupia lithophila* (EEGL).

Biosynthesis of silver nanoparticle composites

The aqueous extract of *Grateloupia lithophila* was obtained by suspending 10 g of finely powdered algal material in 100 mL of double-distilled water, maintained at 110 °C for 15 minutes, filtered, and stored in amber vials to prevent photodegradation. A 2.5 mM silver nitrate stock solution was prepared by dissolving 0.4246 g of $AgNO_3$ in 500 mL of distilled water and incubated in darkness for 24 hours. For nanoparticle synthesis, equal volumes (25 mL each) of the extract and $AgNO_3$ solution were combined under magnetic stirring at 50-60 °C, where a visible colour transition from pale yellow to dark brown signified the biogenic reduction of Ag^+ ions to AgNPs [21, 29, 6, 2, 12, 14].

Separation of silver nanoparticles

The resultant nanoparticles were harvested by centrifugation at 12,000-14,000 rpm for 20 minutes. The supernatant is discarded, and pellet was rinse with double distilled water again centrifuged and dried in hot air oven. After drying the pellet I crushed into fine powder for further assay and stored in ambered colour bottle at room temperature. The synthesized AgNPs-AEGL were subsequently characterized through visual assessment, UV-Visible spectroscopy, SEM, and FTIR analyses, confirming their formation, morphology, and surface functionalization.

Characterization of synthesized AgNP - AEGL

The characterization of synthesized AgNP - AEGL was carried out by using the following analytical parameters

- Visual analysis
- UV- Visible spectral analysis
- Morphological studies using SEM
- FTIR for unctional groups on the surface of $AgNP_S$

Visual examination

Colour change from light to dark brown colour was observed periodically.

UV - Visible spectroscopy

The formation and completion of silver nanoparticles was characterized by UV - Visible spectroscopy by using shimadzu UV- Visible spectrophotometer, model 1800. The bioreduction of the Ag+ ions in solution was monitored by periodical sampling for aliquots and UV - Visible spectra of these aliquots were recorded at 200-600nm range operated at a resolution of 1nm. Distilled was was used as a blank.

Morphological studies of synthesized AgNP-AEGL by uusing Scanning Electron Microscopy (SEM)

Carl zeiss microscopy gmbh Germany (UK)
Model: evo 18
Resolution: 3nm
Magnification: 1× to 1000000×
Operating modes: high vaccum and variable pressure.
Detectors: Qorum technologies.

Anti-Gastric carcinoma study: The assay was carried out by using (3-(4,5- dimethyl thiazol- 2yl)-2,5-diphenyl tetrazolium bromide(MTT). A tetrazolium salt has been used to develop a quantitative colorimetric assay for cell survival and proliferation. The assay detects living, but not dead cells therefore, this method can be used to measure cytotoxicity, proliferation or activation. Cytotoxicity of the given sample was determined against cancer cell lines at 24hours based on MTT colorimetric assay MTT is cleaved by mitochondrial succinate deydrogenase and reductase of viable cells, yielding a measurable purple product formazan. This formazn production is directly proportional to the viable cell number and inversely proportional to the degree of cytotoxicity. Briefly, trypsinized cell were selected in 96 well plate and incubated at 37ºC for 24hr. After 24 hours incubation, the AGS cells were treated with both EEGL AND AgNPs - AEGL in different concentrations for 24 hours. At the end of the treatment the media with drug was discarded and 20 µL of freshly prepared PBS buffered MTT [5 mg/mL] was added to each well and incubated for 4 h in CO2 incubator, after this, the plate was dried by removing the liquor from the well and the formazan formed was carefully dried and dissolved with 100µL of DMSO. Absorbance was recorded on a microplate reader using test wavelength of 570 nm and reference wavelength of 630nm. Final concentration of 0.1% DMSO was usd as negastive control in all experiments. About 100mg of EEGL and AgNPs - AEGL were mixed separately with sterile DMEM media and further dilued to produce the desired concentration [16, 20, 46, 23].

The experimental investigation was carried out over a duration of 14 days employing AGS gastric carcinoma epithelial cell lines, obtained from the National Centre for Cell Science (NCCS), Pune, India, and maintained in Dulbecco's Modified Eagle Medium (DMEM) enriched with L-glutamine, 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin under standard culture conditions.

Sequel

Formulation and evaluation of 2.5mm silver nanoparticles

Green synthesis of silver nanoparticles

Visual Examination

Fig 1: Aqueous solution of 2.5mM AgNO3 with AEGL after 24 hours

Green synthesized silver nanoparticles from EEGL were incubated for the period of 5 hours ad it was periodically examined visually. The colour was changed from light yellowish brown to dark brown, which confirmed that

reduction of Ag+ ions to the silver nanoparticles. Varying colour including yellow, brown, dark brown, brownish black has been reported by several authors. The observations were tabulated and reported. The values are represented in table no. and illustrated in Fig.no 1

The synthesized nanoparticles were separated by centrifugation follwed by drying at hot air oven.

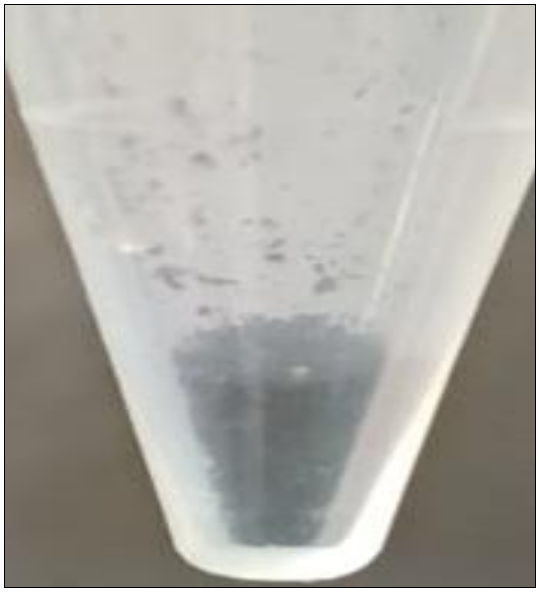

Fig 2: AgNPs - AEGL

Standard graph of silver nanoparticle

AgNP - AEGL was estimated using UV spectrophotometric method by measuring the absorbance at 430nm using deionized water. It obeyed the Beer's lamberts law in the range of 1-5µg/ml. the correlation was found to be 0.998.

Table 1: Standard graph of AgNP - AEGL

S.NO	Concentration(µg/ml)	Absorbance at 430nm
1	1	0.1246
2	2	0.2337
3	3	0.3542
4	4	0.4739
5	5	0.5924

UV Spectrum

The formation of silver nanoparticles was monitored based on plasmon resonance effect of UV light on nano particles. The development of colour in the solution was due to the formation of silver nanoparticles which was attributed to the characteristic surface plasmon vibration of the respective nanoparticles. The absorption maxima was recorded at 420nm due to the presence of surface plasmon resonance. It confirmed the reduction of silver ions to silver nanoparticles. The presence of this peak confirms nanoparticle formation and indicates their colloidal stability in aqueous medium.

Functional group characterization by FT-IR

Table 2: Characterization of AgNP- AEGL

Materials	Functional group	Type of vibration	Characteristic vibration (cm^{-1})	Test absorption(cm^{-1})
Silver nano particles	O-H	Stretching	3310 - 3425	3413.00
	C-H	Stretching	2920 - 2850	2826.19
	C≡C / C≡N	Stretching	2450 - 2530	2053.77
	C=O	Stretching	1740 - 1710	1651.85
	N-H	Bending	1560 - 1500	1540.45
	C-N / COO^-	Stretching	1450 - 1400	1411.08
	S=O / C-N	Stretching	1360 - 1250	1290.86
	C-O-C	Stretching	1230 - 1150	1111.25
	C-O-S / S=O	Bending	900 - 700	860.83
	Ag-O	Bending	700 - 400	613.66

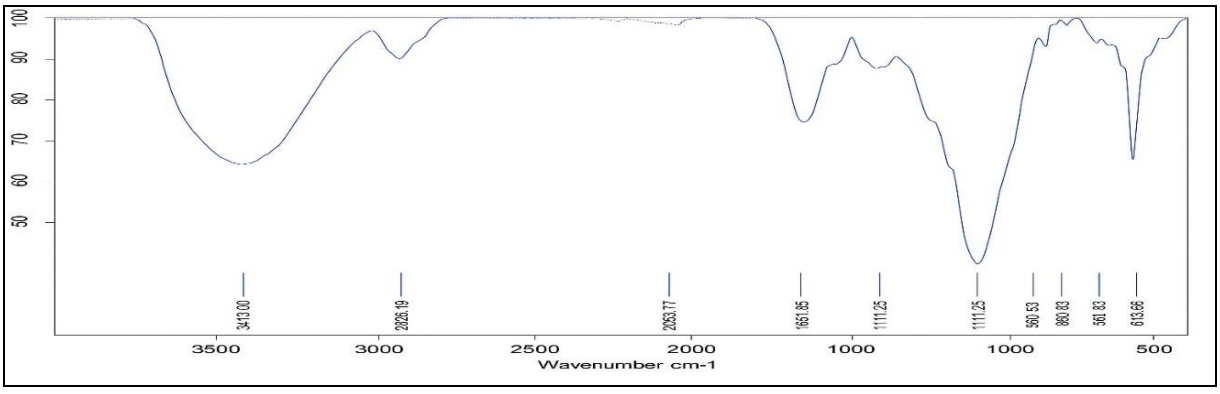

Fig 3: functional group characterization by FT-IR

Morphological studies of agnp-aegl

SEM

SEM analysis was employed to analyse the morphology and size details of the silver nanoparticles that were formed. (fig.4) showed that the silver nanoparticles formed were spherical in shape, with an average size of around 50nm and uniformly distributed silver nanoparticles on the surface of the cells was observed. This confirmed that the phytoconstituents in the AEGL acts as a reducing and capping agent in the synthesis of silver nanoparticles (AgNP-AEGL)

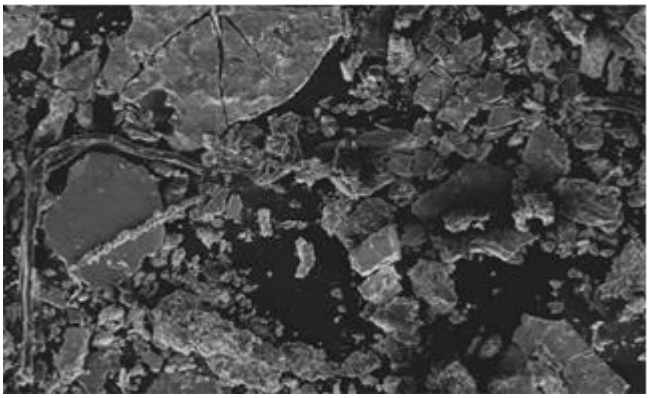

Fig 4: SEM images of AgNP-AEGL

a. 50nm

The aqueous extract of *Grateloupia lithophila* (AEGL) facilitated the synthesis of silver nanoparticles using a 2.5 mM silver nitrate solution. The reaction mixture showed a distinct colour change from light yellowish-brown to dark brown within five hours of incubation. The nanoparticles were separated by centrifugation and dried in a hot-air oven, yielding a fine dark black powder designated as AgNPs-AEGL. Sequential colour transitions were recorded at zero mins, 5mins, 5 hours and 24 hours and are presented in Figure no.4. UV-Visible spectrophotometric analysis of AgNP-AEGL displayed a sharp absorption peak at 420 nm. The absorbance values ranged from 0.1246 to 0.5924 for concentrations between 1 and 5 µg/mL, exhibiting a linear correlation with an R^2 value of 0.998, as shown in Table 1. The FT-IR spectrum of AgNP-AEGL exhibited absorption bands at 3413.00 cm^{-1}, 2826.19 cm^{-1}, 2053.77 cm^{-1}, 1651.85 cm^{-1}, 1540.45 cm^{-1}, 1411.08 cm^{-1}, 1290.86 cm^{-1}, 1111.25 cm^{-1}, 860.83 cm^{-1}, and 613.66 cm^{-1}, corresponding to characteristic vibrational frequencies listed in Table 2 and illustrated in Figure 3. Scanning Electron Microscopy (SEM) revealed that the synthesized nanoparticles were spherical, uniformly distributed, and exhibited an average particle size of approximately 100 nm. The nanoparticles appeared well dispersed with minimal aggregation, as observed in the micrographs were displayed in Figure 4. These findings substantiate the successful green formulation of stable silver nanoparticles from *Grateloupia lithophila* using a 2.5 mM $AgNO_3$ solution, as evidenced through visual observation, UV-Visible spectroscopy, FT-IR spectral characterization, and SEM morphological analysis.

Pharmacological studies

***In vitro* anti- cancer studies against ags cell lines via mtt assay**

In vitro anticancer activity of both EEGL and AgNPs-AEGL was performed against AGS cell lines. The cell lines were grouped as control, standard, EEGL treated and AgNPs-AEGL treated. The percentage cell viability and cytotoxicity was determined by reading the absorbance at 570 and 630nm were presented in table no.3 & 4.

Table 3: Percentage of cell viability

s.no	Concentration (µg/ml)	% cell viability mean± SEM		
		5-FU	EEGL	AgNPs-AEGL
1	6.25	88.186 ± 1.864	93.3106817 ± 0.3704	87.39086 ± 0.003638
2	12.5	73.111 ± 2.732	91.2295604 ± 0.5344	74.31475 ± 0.001015
3	25	52.643 ± 1.237	83.7332767 ± 0.4434	56.95142 ± 0.000585
4	50	31.547 ± 0.193	73.773625 ± 0.6383	38.12863 ± 0.000467
5	100	16.817 ± 0.377	35.1242302 ± 1.6761	27.48166 ± 0.000586
	IC50	25.93	76.43	34.23

Table 4: Percentage of cell cytotoxicity

s.no	Concentration (µg/ml)	% cytotoxicity mean± SEM		
		5-FU	EEGL	AgNPs-AEGL
1	6.25	11.814 ± 1.076	6.689318 ± 0.370261	12.60914 ± 0.18957
2	12.5	26.889 ± 1.577	8.77044 ± 0.534285	25.68525 ± 0.16491
3	25	47.357 ± 0.714	16.26672 ± 0.443419	43.04858 ± 0.35773
4	50	68.453 ± 0.111	26.22638 ± 0.638139	61.87137 ± 0.19145
5	100	83.183 ± 0.217	64.87577 ± 1.675218	72.51834 ± 0.36556
	IC50	25.93	76.43	34.23

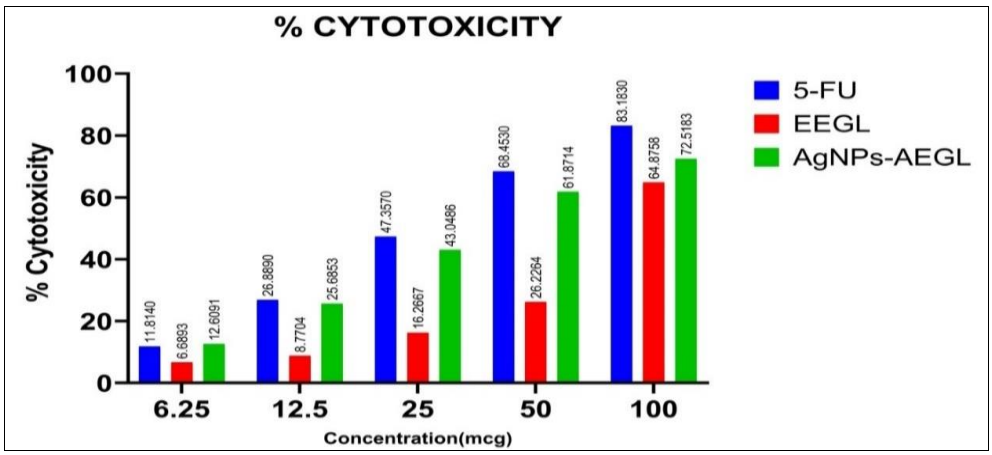

Fig 5: % Cell cytotoxicity graph of 5-FU, EEGL and AgNPs-AEGL

Table 5: Variable slope Dose-Response Parameters for 5-FU, EEGL and AgNPs-AEGL

log(inhibitor) vs. normalized response - Variable slope		Drug Therapy		
		5-FU	EEGL	AgNPs-AEGL
Best-fit values	LogIC50	1.41380	1.883	1.535
	HillSlope	0.98	1.664	1.047
	IC50	25.93	76.43	34.23
Std. Error	LogIC50	0.0621	0.02451	0.058
	HillSlope	0.12	0.1682	0.089
95% Confidence Intervals	IC50	1.2962 to 1.5314	1.830 to 1.936	1.421 to 1.649
	HillSlope	0.7448 to 1.2152	1.301 to 2.027	0.873 to 1.221
	IC50	19.779 to 33.994	67.66 to 86.35	31.25 to 37.21
Goodness of Fit	Degrees of Freedom	11	13	11
	R^2	0.9671	0.9471	0.997
	Absolute Sum of Squares	222.75	361.0	250.01
	Sy.x	4.50	5.269	4.7673
Number of points	Analyzed	15	15	15

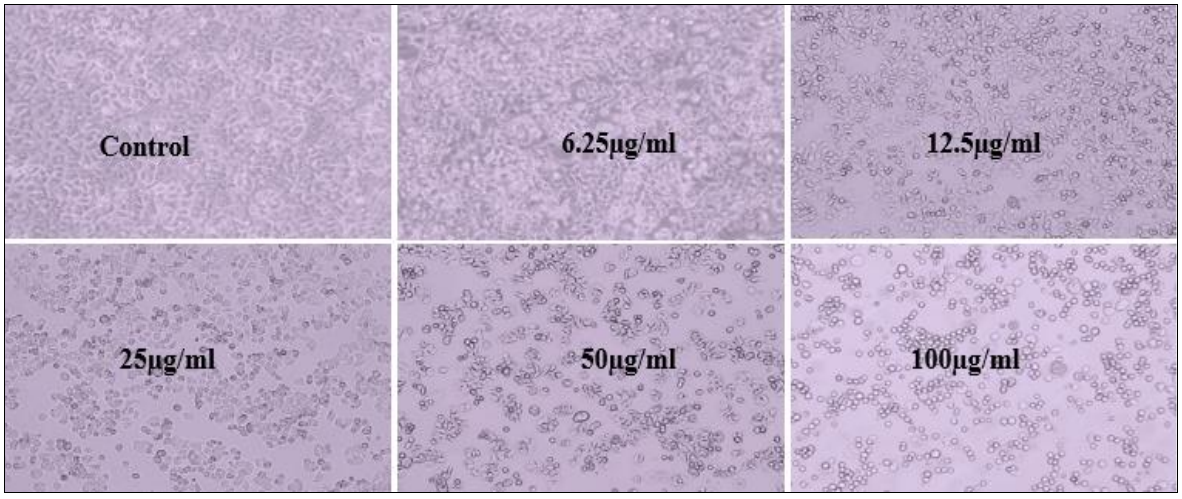

Fig 6(a): MTT assay images of EEGL

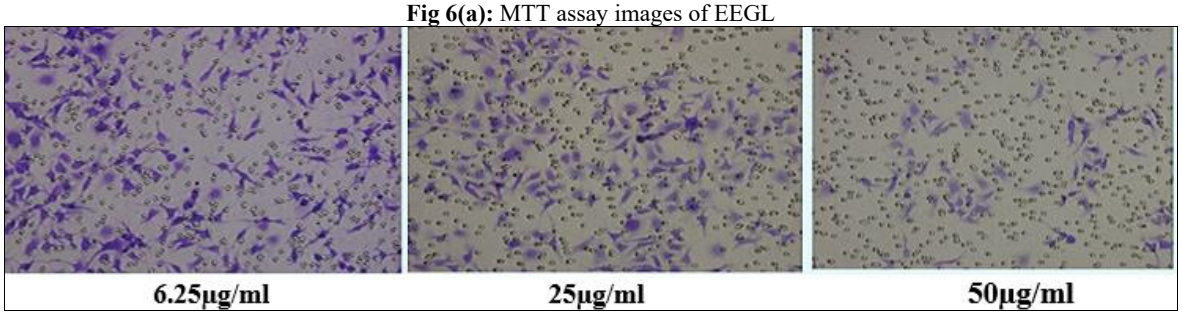

Fig 6(b): MTT assay images of AgNPs -AEGL

The *in vitro* cytotoxicity of ethanolic extract of *Grateloupia lithophila* (EEGL) and silver nanoparticles synthesized using the aqueous extract of *G. lithophila* (AgNPs-AEGL) was evaluated against AGS human gastric adenocarcinoma cell lines. The cells were divided into four groups such as untreated control, standard drug (5-Fluorouracil), EEGL-treated, and AgNPs-AEGL-treated. Cell viability was assessed using the MTT assay at concentrations of 6.25, 12.5, 25, 50, and 100 µg/mL.

The percentage of viable cells decreased in a dose-dependent manner in all treatment groups. For 5-FU, cytotoxicity increased from 11.81% at 6.25 µg/mL to 83.18% at 100 µg/mL. EEGL exhibited cytotoxicities of 6.69%, 8.77%, 16.27%, 26.23%, and 64.88% at concentrations of 6.25, 12.5, 25, 50, and 100 µg/mL, respectively. AgNPs-AEGL showed cytotoxicities of 12.61%, 25.69%, 43.05%, 61.87%, and 72.52% at the corresponding concentrations. The calculated IC_{50} values were 25.93 µg/mL for 5-FU, 76.43 µg/mL for EEGL, and 34.23 µg/mL for AgNPs-AEGL.The log(inhibitor) versus normalized response analysis revealed $LogIC_{50}$ values of 1.414 for 5-FU, 1.883 for EEGL, and 1.535 for AgNPs-AEGL. The Hill slopes were 0.98, 1.664, and 1.047, respectively. Goodness-of-fit parameters indicated R^2 values of 0.9671 for 5-FU, 0.9471 for EEGL, and 0.997 for AgNPs-AEGL. The 95% confidence intervals for IC_{50} ranged from 19.779-33.994 µg/ml for 5-FU, 67.66-86.35 µg/ml for EEGL, and 31.25-37.21 µg/ml for AgNPs-AEGL. Degrees of freedom were 11, 13, and 11, with an absolute sum of squares of 222.75, 361.0, and 250.01 and Sy.x values of 4.50, 5.269, and 4.7673, respectively. Fifteen data points were analyzed for each treatment.Representative MTT assay images for 5-FU, EEGL, and AgNPs-AEGL at varying concentrations are presented in Fig.9. The purple formazan staining diminished progressively with increasing concentrations, reflecting a reduction in viable cells. While

EEGL caused partial cell death even at higher concentrations, AgNPs-AEGL induced marked cytotoxicity at relatively lower concentrations, consistent with the observed IC_{50} values. Collectively, these results indicate that AgNPs-AEGL possesses potent anticancer activity against AGS cells, surpassing the effect of the crude extract and approaching the efficacy of the standard chemotherapeutic agent 5-FU. This underscores the potential of green-synthesized silver nanoparticles as an effective delivery system to enhance the bioactivity of *G. lithophila* phytoconstituents.

Deliberation

The present study focused on the green synthesis of silver nanoparticles (AgNPs) using the aqueous extract of *Grateloupia lithophila* (AEGL), selected deliberately over other conventional dosage forms due to the superior bioavailability, targeted delivery, and enhanced pharmacological activity offered by nanoparticles. It can efficiently penetrate cellular membranes, improve solubility of bioactive compounds, and provide controlled release, making them particularly suitable for biomedical applications, including cancer therapy. The selection of aqueous extract over ethanolic extract (EEGL) was intentional and scientifically justified. Aqueous extracts typically retain a higher concentration of polar biomolecules such as polysaccharides, flavonoids, phenolics, and proteins that exhibit strong reducing and capping capabilities, which are essential for nanoparticle nucleation, stabilization, biocompatibility, and cellular permeability. Conversely, ethanolic extracts, although rich in semi-polar compounds, may lose some thermolabile or highly polar constituents critical for controlled nanoparticle formation. Hence, the aqueous extract provided an optimal phytochemical matrix to facilitate consistent reduction of silver ions and to ensure nanoparticle stability without additional chemical stabilizers [40, 42, 38]. Moreover, silver nanoparticles themselves exhibit intrinsic cytotoxic properties through reactive oxygen species generation, DNA damage, and induction of apoptosis in cancer cells. By formulating the bioactive compounds from *G. lithophila* into AgNPs, the therapeutic potential of the algal metabolites is synergistically combined with the advantages of nanotechnology, enhancing efficacy and stability [35].

In our findings the phytoconstituents present in AEGL play a dual and critical role in both the formulation process and therapeutic potential. Alkaloids, flavonoids, phenolics, polysaccharides, proteins, and terpenoids act as natural reducing agents, facilitating the conversion of silver ions (Ag^{+}) into elemental silver (Ag^{0}), and simultaneously function as capping and stabilizing agents, preventing aggregation and ensuring uniform particle morphology. These same bioactive molecules contribute to the potential anticancer properties of the nanoparticles, highlighting the significance of selecting a phytochemical rich extract for nanoparticle synthesis.Evaluation of the formulated nanoparticles is essential to confirm successful synthesis, stability, and functional relevance. In the current study, visual observation revealed sequential color changes from light yellowish-brown at initiation to dark brown over 24 hours, indicating continuous reduction and growth of silver nanoparticles. The final black powder obtained after drying represents a stable and reproducible product. This progressive color change, attributed to surface plasmon resonance (SPR), is a novel observation for *G. lithophila*, as previous studies on related red algae have reported brownish powders rather than deep black powders. The final dark coloration reflected the completion of the reduction process and the formation of stable nanoparticles [43, 1].

UV-Visible spectroscopy further confirmed nanoparticle formation, with AgNPs-AEGL displaying a sharp SPR peak at 420 nm, characteristic of spherical nanoparticles with uniform size distribution. The absorbance values ranged from 0.1246 to 0.5924 for concentrations between 1-5 µg/mL, with a correlation coefficient (R^2) of 0.998, demonstrating linearity according to Beer-Lambert's law. This indicates a uniform particle population and minimal aggregation [19, 32]. Compared to previous algal-mediated nanoparticle studies, the slightly higher intensity of the SPR peak suggests a denser nanoparticle formation, which has not been previously reported for *G. lithophila*, representing a novel finding. Fourier Transform Infrared (FT-IR) spectroscopy provided insight into the molecular interactions responsible for nanoparticle formation and stabilization. The prominent peaks at 3413 cm^{-1} (O-H and N-H stretching), 1651 cm^{-1} (C=O stretching of amide I), and 1411 cm^{-1} (C-O symmetric stretching of carboxylate groups) confirmed the involvement of hydroxyl, amide, and carboxylate functionalities in nanoparticle stabilization. Additional bands at 1111 cm^{-1} and 613 cm^{-1} corresponded to polysaccharide vibrations and Ag-O coordination, respectively, affirming the binding of phytochemicals to the nanoparticle surface. These findings indicate that polysaccharides, proteins, and phenolic compounds in AEGL act synergistically as natural reducing and stabilizing agents [35, 42, 38]. The FT-IR data thus validate that AEGL biomolecules not only facilitated silver ion reduction but also ensured colloidal stability through surface functionalization. Notably, the detailed assignment of FT-IR bands for *G. lithophila*-mediated AgNPs is novel, as previous studies on related algae did not report the coordination patterns with silver ions in such detail.

Scanning Electron Microscopy (SEM) analysis revealed that the synthesized AgNPs-AEGL were predominantly spherical in morphology with an average particle size ranging from 1 to 50 nm. The uniformity in shape and size distribution confirmed controlled nucleation and effective capping by the phytochemicals present in the aqueous extract. The absence of significant aggregation further indicated strong stabilization of nanoparticles, supporting the hypothesis that AEGL constituents act as efficient natural surfactants. The morphological observations are consistent with those reported in similar green synthesis studies, where plant or algal extracts produced spherical, monodispersed nanoparticles due to the presence of abundant hydroxyl and carbonyl groups [26, 24].

The success of the synthesis process can be attributed to the synergistic action of multiple phytoconstituents present in *G. lithophila*, including flavonoids, alkaloids, polysaccharides, terpenoids, and phenolic compounds. These biomolecules facilitate the transfer of electrons required for the reduction of Ag^{+} to Ag^{0} and simultaneously form protective coatings around nanoparticles, thereby preventing oxidation and aggregation. Moreover, the aqueous extract of *G. lithophila* is rich in sulfated galactans and other hydrophilic macromolecules characteristic of red algae, which enhance nanoparticle stability through electrostatic and steric effects. This biochemical complexity

positions *G. lithophila* as an ideal biofactory for green nanotechnology applications.

From a biomedical perspective, the AgNPs-AEGL formulation exhibits promising potential. Silver nanoparticles are known for their broad-spectrum antimicrobial and anticancer activities, primarily mediated through the generation of reactive oxygen species (ROS), mitochondrial dysfunction, and induction of apoptotic pathways [38]. The incorporation of *G. lithophila* bioactives within AgNPs potentially enhances therapeutic efficacy due to synergistic interactions between algal metabolites and silver nanostructures. Furthermore, the nanoscale dimension of AgNPs allows improved cellular uptake, controlled drug release, and enhanced bioavailability, which are advantageous for biomedical and pharmaceutical applications.

Importantly, AEGL-mediated AgNPs may offer significant anti-gastric cancer activity. Flavonoids, polysaccharides, and sulfated galactans present in *G. lithophila* have been reported to induce apoptosis in gastric carcinoma cells, inhibit proliferation, and modulate inflammatory signaling pathways such as NF-κB and PI3K/Akt [37, 25, 44]. When formulated as AgNPs, these bioactives may penetrate gastric tumor cells more efficiently due to nanoscale size, enhancing ROS-mediated cytotoxicity and selective tumor targeting. Additionally, the antioxidant and immunomodulatory properties of algal phytochemicals can contribute to nutraceutical applications, supporting gastrointestinal health and offering preventive benefits against oxidative stress-induced gastric pathologies [31, 34, 18, 7, 45]. Therefore, AEGL-AgNPs represent a dual-purpose formulation combining therapeutic and nutraceutical potential.

The results from visual, UV-Vis, FT-IR, and SEM analyses collectively confirm the successful green synthesis of stable, spherical silver nanoparticles using the aqueous extract of *Grateloupia lithophila*. The findings are consistent with previous algal-mediated nanoparticle studies and demonstrate that *G. lithophila* provides a sustainable, eco-friendly source of biomolecules capable of efficiently reducing and stabilizing silver nanoparticles. These bio-fabricated AgNPs exhibit properties that make them promising candidates for biomedical and pharmacological applications.

The in-*vitro* anticancer assessment of *Grateloupia lithophila* against AGS human gastric adenocarcinoma cells demonstrated a distinct and concentration-dependent cytotoxic response for both the ethanolic extract of *Grateloupia lithophila* (EEGL) and its green-synthesized silver nanoparticle formulation of aqueous extract of *Grateloupia lithophila* (AgNPs-AEGL). The AGS cell line was judiciously selected as a representative *in vitro* model owing to its authentic gastric epithelial origin, rapid proliferative capacity, and established utility in preclinical screening of anti-stomach cancer agents, thereby providing a clinically pertinent platform for evaluating novel phytochemical based therapeutics.The standard chemotherapeutic drug 5-Fluorouracil (5-FU) was employed as the reference control because of its well-documented efficacy and mechanism of action involving thymidylate synthase inhibition and disruption of DNA replication [35]. The *in vitro* anticancer evaluation of *Grateloupia lithophila* demonstrated a concentration-dependent cytotoxic effect against AGS human gastric adenocarcinoma cells. The study compared the ethanolic extract (EEGL) and the silver nanoparticle formulation synthesized from the aqueous extract (AgNPs-AEGL) with the standard chemotherapeutic drug 5-fluorouracil (5-FU). Among the tested samples, AgNPs-AEGL exhibited significantly enhanced cytotoxic potential relative to EEGL, suggesting that nanoparticle formulation effectively improved the bioavailability and cellular interaction of the bioactive metabolites [20].

The MTT assay results revealed a marked reduction in cell viability with increasing concentrations of all test samples. The IC_{50} values were recorded as 25.93 µg/mL for 5-FU, 76.43 µg/mL for EEGL, and 34.23 µg/mL for AgNPs-AEGL, respectively. The lower IC_{50} value of AgNPs-AEGL relative to EEGL indicates a stronger inhibitory effect on AGS cell proliferation, signifying that the green-synthesized silver nanoparticles mediated a higher degree of cytotoxicity. This enhanced efficacy can be attributed to the nanoscale size, which allows easier penetration through the cellular membrane, facilitating efficient intracellular delivery of the phytochemicals and interaction with key molecular targets responsible for tumor growth and survival [19]. At the lowest concentration of 6.25 µg/mL, all test groups showed minimal inhibition of AGS cell proliferation. The percentage cell viability remained high 93.31% for EEGL, 87.39% for AgNPs-AEGL, and 88.18% for 5-FU indicating that only a small portion of the cancer cells were affected. This suggests that at very low concentrations, the intracellular levels of active metabolites or nanoparticles were insufficient to trigger oxidative or apoptotic stress, allowing mitochondrial dehydrogenases to remain functionally active. Cell viability was quantified using the MTT assay, which measures mitochondrial activity through the enzymatic conversion of the tetrazolium dye MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] into insoluble purple formazan crystals by metabolically active cells. The absorbance of solubilized formazan at 570 and 630 nm directly reflects mitochondrial integrity [39]. Untreated control wells exhibited intense purple coloration, denoting maximum cell viability, whereas EEGL and AgNPs-AEGL treated wells showed a progressive attenuation of this coloration with increasing concentration, indicating inhibition of mitochondrial enzyme activity and suppression of cellular metabolism. At 100 µg/mL, the AgNPs-AEGL treated cells exhibited a markedly diminished purple intensity compared with EEGL and control groups, visually substantiating its pronounced cytotoxic potential [5, 27].

At the lowest concentration of 6.25 µg/mL, cell viability remained high at 93.31% for EEGL, 87.39% for AgNPs-AEGL, and 88.18% for 5-FU, indicating that minimal cytotoxicity occurs at sub-therapeutic concentrations likely due to insufficient intracellular levels of active metabolites or nanoparticles to induce oxidative or apoptotic stress. Upon increasing the concentration to 12.5 µg/mL, a slight but discernible reduction in cell viability was observed, especially for AgNPs-AEGL (74.31%), which paralleled the cytotoxicity of 5-FU (73.11%). The ethanolic extract, however, maintained a relatively high viability (91.22%), suggesting that the ethanol-soluble constituents alone exert only moderate stress at this concentration [5, 41]. The increased cytotoxicity of AgNPs-AEGL (25.68%) at this point can be correlated with the initial interaction between AgNPs and the negatively charged cancer cell membranes, leading to membrane destabilization and early

mitochondrial dysfunction.At 25 µg/mL, the cytotoxicity markedly intensified for all samples. Viability dropped to 83.73% for EEGL, 56.95% for AgNPs-AEGL, and 52.64% for 5-FU. The nanoparticle formulation thus surpassed the ethanolic extract and nearly matched the reference drug, signifying effective intracellular accumulation and possible initiation of ROS generation. The nanoparticles likely facilitated deeper penetration through endocytosis, leading to oxidative damage and activation of caspase-dependent apoptotic pathways [39, 20]. At 50 µg/mL, there was a pronounced cytotoxic effect with only 38.12% viable cells for AgNPs-AEGL, 31.55% for 5-FU, and 73.77% for EEGL. The drastic drop in viability for AgNPs-AEGL indicates mitochondrial collapse and DNA fragmentation, typical of advanced apoptosis. The ethanolic extract, though less potent, showed a considerable reduction in viability, suggesting cumulative intracellular stress that might activate cell cycle arrest at the G_2/M phase. The data at this level mark the threshold where sustained oxidative stress overrides cellular antioxidant defenses.

At the highest tested concentration (100 µg/mL), the cytotoxicity reached its maximum, with 16.81% viable cells for 5-FU, 27.48% for AgNPs-AEGL, and 35.12% for EEGL. The sharp decline in metabolic activity, reflected by low absorbance readings, indicates nearly complete inhibition of mitochondrial dehydrogenase activity and severe disruption of cellular architecture. Microscopic images revealed significant cytomorphological aberrations such as cellular pyknosis, cytoskeletal reorganization, plasma membrane vesiculation, and anoikis, all characteristic of apoptotic and necrotic pathways [20].

Furthermore, the dose-response curve fitting demonstrated high correlation coefficients (R^2 = 0.9671 for 5-FU, 0.9471 for EEGL, and 0.997 for AgNPs-AEGL), confirming the reliability and consistency of the assay. The Hill slope values of 0.98 for 5-FU, 1.664 for EEGL, and 1.047 for AgNPs-AEGL were reflect cooperative binding behavior of the bioactive compounds to cellular targets, with AgNPs-AEGL showing a balanced sigmoidal response indicative of steady inhibitory action across the tested concentrations.

The observed anticancer activity of *Grateloupia lithophila* could be linked to its diverse secondary metabolites, including alkaloids, flavonoids, terpenoids, phenolics, and polysaccharides, all of which possess documented antioxidant and antineoplastic properties [5, 9, 10, 33, 45, 22]. The phytoconstituents likely act through multiple mechanisms such as induction of apoptosis, disruption of mitochondrial membrane potential, inhibition of cell proliferation, and modulation of key signaling pathways involved in cancer cell survival [41, 28, 36]. The reduction in absorbance and increase in cytotoxicity at higher concentrations suggest that both formulations effectively interfere with mitochondrial dehydrogenase activity, confirming the metabolic suppression of malignant cells.

In our findings, The diverse bioactive phytoconstituents of *Grateloupia lithophila* including flavonoids, alkaloids, terpenoids, phenolics, saponins, and fatty acids were identified through preliminary screening, TLC, and GC-MS, play a pivotal role in anticancer activity by orchestrating apoptosis, oxidative stress induction, and cell-cycle modulation in gastric cancer cells. Physicochemical and fluorescence analyses corroborate the integrity and presence of redox-active metabolites, which potentiate ROS-mediated cytotoxicity. Concurrently, heavy metal evaluation ensures biosafety, while essential trace elements and micronutrients synergistically enhance antioxidant defenses, immunomodulation, and cellular repair, collectively reinforcing both the therapeutic and nutraceutical potential of the marine algae.

Grateloupia lithophila qualifies as a nutraceutical due to its convergence of bioactive phytoconstituents, essential nutrients, and biosafe profile. The alga is rich in flavonoids, alkaloids, terpenoids, phenolics, saponins, and fatty acids, which exhibit antioxidant, anti-inflammatory, and cytoprotective activities, collectively supporting cellular health and metabolic regulation [22, 37, 44, 45]. Quantitative analyses reveal significant levels of essential trace elements (Zn, Cu, Fe) and micronutrients including vitamins A, B_1, B_2, and C, which enhance enzymatic antioxidant defense, DNA repair, immune function, and overall physiological resilience. Importantly, heavy metal assessment confirms that toxic metals (Pb, Cd, Hg) are within permissible limits, ensuring safety for consumption. This combination of therapeutic phytochemicals and nutritional value, alongside demonstrated cytotoxic efficacy against cancer cells, provides a robust scientific rationale for considering *G. lithophila* as a marine nutraceutical capable of delivering both preventive and health-promoting benefits [9, 10, 33, 41].

The superior performance of the AgNPs-AEGL formulation further underscores the importance of nanotechnology in natural product-based drug development. Silver nanoparticles act not only as carriers but also as active agents capable of generating reactive oxygen species (ROS), leading to oxidative stress-mediated apoptosis. The synergy between phytochemicals from *G. lithophila* and the metallic nanoparticles likely contributes to improved drug targeting, controlled release, and amplified cytotoxicity. These findings align with previous reports where silver nanoparticle-mediated formulations exhibited enhanced anticancer properties compared to their crude extracts, owing to their increased surface area-to-volume ratio and bio-interactive nature [19, 46, 23].

In comparison with the reference drug 5-fluorouracil, which exhibited the most potent cytotoxicity, the AgNPs-AEGL formulation demonstrated a remarkably close inhibitory profile, indicating its potential as a natural therapeutic alternative with reduced systemic toxicity. Conversely, the relatively higher IC_{50} value of EEGL suggests that while the ethanolic extract contains bioactive components, their limited solubility and lower cellular uptake may restrict their cytotoxic efficiency. Therefore, nanoparticle encapsulation represents a promising strategy to enhance the pharmacological performance of *Grateloupia lithophila* constituents in anticancer therapy.

Intrestingly, the results establish that *Grateloupia lithophila* possesses significant anticancer potential against AGS gastric carcinoma cells, with its nanoparticle formulation exhibiting superior cytotoxic potency. These findings support the further exploration of *G. lithophila*-derived nanotherapeutics as a viable approach in developing alternative or adjunct therapies for gastric cancer.

Conclusion

The present study underscores *Grateloupia lithophila* as a compelling marine algal source of bioactive compounds exhibiting pronounced cytotoxic efficacy against AGS gastric carcinoma cells. Both the ethanolic extract and its biogenically synthesized silver nanoparticles demonstrated

potent, dose-dependent antiproliferative activity, with the nanoformulation displaying enhanced therapeutic performance comparable to 5-fluorouracil. These findings collectively advocate the translational potential of *G. lithophila*-derived phytoconstituents, particularly in nanoparticle form, as innovative and sustainable candidates for gastric cancer intervention.

Future perspectives

Future studies should focus on elucidating the molecular mechanisms and *in vivo* efficacy of *Grateloupia lithophila*-derived silver nanoparticles. Integrating omics, molecular docking, and pharmacokinetic analyses will clarify their therapeutic pathways and safety. Optimization of large-scale green synthesis and targeted delivery strategies could establish AgNPs-AEGL as a potent, eco-sustainable nanotherapeutic for gastric cancer treatment.

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