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Research Article

PHYTOCHEMICAL SCREENING AND DETERMINATION OF ANTIMICROBIAL, ANTIOXIDANT, AND ANTICANCER ACTIVITY OF *ULVA LACTUCA*-BASED SILVER NANOPARTICLE

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ABSTRACT

Objective: The current study is proposed to evaluate the potential medicinal value of *Ulva lactuca* an edible green seaweed. The prime objectives of the research were to determine the anti-oxidant, anti-microbial, and anti-cancer properties of the seaweed extracts and green synthesized nanoparticles.

Methods: Five different solvent extracts were qualitatively and quantitatively analyzed for phytochemicals. A gas chromatography-mass spectrophotometer (GC-MS) analyzed the metabolite profile of the methanol extract. *In-vitro* anti-oxidant activity is determined by 1-diphenyl 2-picrylhydrazyl (DPPH) and ABT assay. The Resazurin method tested the anti-microbial activity against two uropathogenic bacteria and one fungal pathogen. HeLa cell line was employed to investigate the anti-cancer potential of the seaweed conjugated nanoparticle.

Results: Qualitative analysis revealed the presence of Alkaloids, Phenol, flavonoids, tannins, steroids, carbohydrates, glycosides, amino acids, and proteins. The metabolite profiling of methanol extract was identified by GC-MS analysis. Quantitative estimation exposed total flavonoid content of 2.56±0.30 mg quercetin equivalent/g, total phenolic content −3.66±0.15 mg gallic acid equivalents/g, Tannic acid equivalent – total tannin content (TTC) of 2.90±0.61 mg/g and 3.40±0.30 mg/dL of steroids. EAE, ME, and HE recorded the following IC_{50} for DPPH −871 µg/mL, 432.264 µg/mL, and 432.273 µg/mL, respectively. In ABTs, AE, ME, and EAE showed the highest activity at IC_{50} values of 39.090 µg/mL, 104.43 µg/mL, and 252.491 µg/mL. MIC of *Ulva* NP against *Escherichia coli* −250 µg/mL, *Candida albicans* −500 µg/mL, and *Acinetobacter baumannii* −1000 µg/mL was depicted. The cytotoxicity nature of UAgNPs is observed in HeLa cell lines. The screening results reveal that the edible green seaweed *U. lactuca* can be further studied and extended as a potential source of components in controlling Urinary tract infection (UTI) and a drug of choice for cervical cancer.

Conclusion: The current study highlights the antimicrobial, antioxidant, and anticancer properties of green seaweed *U. lactuca*, a potential source of pharmaceutical application.

Keywords: *Ulva lactuca*, Green nanoparticle, HeLa, MIC, MTT assay, Anti-oxidant activity, Anti-cancer activity, Resazurin test.

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INTRODUCTION

Urinary infection is the most common transmittable disease among the population worldwide.

Diverse etiological agent, bacterial – *Escherichia coli*, *Klebsiella* species, *Pseudomonas* species, *Proteus* species, and the most common fungal pathogen *Candida albicans*, are responsible for UTI in young children to all ages adults, especially diabetic women [1]. The extensive use of antibiotics has developed antimicrobial resistance among microorganisms, one of the top 10 global public health threats facing humanity, as declared by the World Health Organization. *Enterobacteriaceae* and *Acinetobacter baumannii* [2] AMR strains are considered critical pathogens that need to be controlled by an alternative to antibiotics in an eco-friendly way. Cancer remains a nightmare globally, affecting millions of people of varying ages and cultures. Chemotherapy is one of the conventional treatments given extensively to control cancer. Using nanoparticles as drug carriers and improving the drug's bioavailability shows a new array of light in controlling many disease conditions, including cancer [3]. Nanoparticle synthesis and its application is a universal breakthrough. Green synthesis (biogenesis) is one of the most promising approaches for producing size-controlled nanoparticles [4]. Nanotechnology has many applications, in various fields, such as medicine, agriculture, cosmetics, and the environment. The biological extracts employed in the synthesis of nanoparticles are more appropriate because they are economical, non-toxic, safe, and eco-friendly [5]. Ag NPs are the traditional and most desired target of green approaches, being employed in the textile industry, agriculture, water treatment, and other fields, as well as for medical purposes. Recently, nanoparticles were applied as a biodiesel additive [6]. Pharmacognosy-drug discovery using natural products, such as medicinal plants or marine algae remains an important target in current research [7]. Seaweeds are significant natural resources from the marine habitat and they are rich in beneficial chemical components. Macroalgae is classified into three phyla: Chlorophyta (Green), Rhodophyta (Red), and Phaeophyta (Brown). Red and brown seaweeds are extensively studied for their bioactive dimensions. Seaweeds play a vital part in the production of new compounds [8], used as animal feed [9], and as pre-biotics in the food industry [10]. The bioactive components have numerous applications and are in high demand due to their various bioactivities of therapeutic significance, such as anti-bacterial, anti-viral, anti-fungal, anti-cancer [11], anti-diabetic [12], anti-inflammatory [13], and anti-oxidant properties [14,15]. Chye *et al.* [16] stated that seaweeds have applications in the food, pharmaceutical, medical, cosmetic, and agricultural sectors. Seaweeds are also used as a supplement in traditional food cultures to overcome nutritional deficiencies [17], and for extracting or isolating bioactive substances to formulate nutraceutical supplements. Seaweeds could potentially aid in managing many health issues [18]. The present study concentrated on Sea lettuce *Ulva lactuca*, edible green seaweed that grows up to 7–12 inches. It is a thin, flat green algae growing from a holdfast and belongs to the family *Ulvaceae* in

the Phylum *Chlorophyta*. The current study mainly focused on the anti-oxidant, anti-microbial, and anti-cancer activities of seaweed conjugated silver nanoparticles and the *U. lactuca* phytochemical components screening.

METHODS

Materials
Analytical grade chemicals, solvents, and media employed were procured from Merck, Sigma, and Hi-media.

Sample collection and processing
Live Green Seaweed *Ulva species* were collected from the coastal region of Mandapam, Ramanadhapuram, Tamil Nadu. The samples were thoroughly washed with seawater to remove the surface debris, and rinsed with distilled water. The cleansed seaweed was shade-dried, powdered, and stored for further extraction. The seaweed was identified as *U. lactuca* by the authenticated botanist in the Botanical Survey of India, Howrah, India (Fig. 1).

Preparation of extract and characterization
The five different solvent extracts of *U. lactuca* were prepared by employing: Chloroform, Ethylacetate, Hexane, Methanol, and Aqueous with varying polarity in Soxhlet at the proper temperature. The extracts were concentrated, dried, and stored at 40°C.

Characterization
The extracts were examined for phytochemical components.

Qualitative analysis of phytochemicals
The standard qualitative Phytochemical analysis was performed for the following components - Alkaloids, Terpenoids, Steroids, Phenol, Flavonoid, Tannin, Carbohydrate, Saponin, and Glycosides [19-23] from the solvent extracts.

Quantitative analysis of phytochemicals estimation of total phenolic content (TPC)
The Folin-Ciocalteau method is used to determine the TPC of the methanol extract [24]. An aliquot of 0.1 mL of seaweed extract was mixed with 3 mL of distilled water, and 0.5 mL of Folin-Ciocalteau reagent was added. To this, 20% sodium carbonate is added and mixed thoroughly. The tubes were incubated in a boiling water bath for 30 min and then cooled, and absorbance was measured at 760 nm. Total phenol concentration was estimated by a standard calibration curve using different concentrations of standard gallic acid (0.01–0.1 mM), and the results were expressed as mg of gallic acid equivalents (GAEs) per g of extract.

Fig. 1: Green seaweed *Ulva lactuca*

Estimation of total flavonoid content (TFC)
TFC of methanol extracts of seaweed was estimated using the aluminum chloride method, as stated by Kamtekar *et al*. [25]. About 0.5 ml of 2% $AlCl_3$ in an ethanol solution was added to 0.5 mL of extract, after an hour of incubation at room temperature, the development of the yellow color was observed. With a ultraviolet (UV)-visible spectrophotometer, absorbance was measured at 420 nm. A standard graph is prepared using quercetin, and the TFC is expressed in mean as quercetin equivalent (mg QE/g).

Estimation of steroids
The ferric chloride method [26] was employed to estimate steroids in the extract. $Fecl_3$ 4.9 mL was added to 0.1 mL of seaweed extract and centrifuged. An equal quantity of supernatant (2.5 mL) was added to 2.5 mL of ferric chloride diluting agent and 4.0 mL of concentrated sulfuric acid. A blank was prepared simultaneously with 5.0 mL of diluting reagent and 4.0 mL of concentrated sulfuric acid. A set of standards (0.5–2.5 mL) was taken and made up to 5.0 mL with ferric chloride diluting reagent and 4.0 mL of concentrated sulfuric acid. After 30 min, the intensity of color was read at 540 nm against a reagent blank. The quantity of Steroids in the sample is expressed as mg/dL.

Estimation of tannin content
The Folin-Denis method is used to estimate Tannin. Standard tannic acid solution (0.2–1.0 mL) was taken in a set of test tubes, and 0.5 mL of extract solution in another test tube was taken. The volume of all the tubes was increased to 3 mL with distilled water. Distilled water alone was taken as blank. To all the tubes, 5 mL of 35% Na_2CO_3 and 2.5 mL of Folin-Denis reagent are added and incubated at room temperature for 30 min. The absorbance reading against the reagent blank is read at 700 nm. From the standard graph, the amount of Tannin present in the sample was expressed as mg of tannic acid equivalent per gram of seaweed extract [27].

Gas chromatography-mass spectrophotometer (GC-MS) analysis
The profile of metabolites present in the methanol extract of *U. lactuca* (UMB) was identified by GC-MS analysis. GC-MS Shimadzu-QP2010 plus Model analyzed the sample. The following chromatographic conditions were maintained: Helium gas was used as the carrier at a flow rate of 1.05 mL/min; the injector was operated at 250°C, and the column oven temperature was programmed at 45–280°C at a rate of 10°C/min in injection mode. The MS conditions used were an ionization voltage of 70 eV, an ion source temperature of 200°C, an interface temperature of 280°C, and a mass range of 40–700 m/z. The unknown component spectrum was compared with the mass spectrum of the known components in the National Institute of Standards and Technology (NIST 14s—lib) library.

Anti-oxidant activity
1-diphenyl 2-picrylhydrazyl (DPPH) radical scavenging activity

The antioxidant activity of ultrasonic-assisted extraction (UAE), ultrasound-enzyme-assisted extraction (UEAE), UHE, Ulmus macrocarpa Hance extract (UME), and UCE was measured based on the scavenging activity of the stable 1, DPPH free radical following the modified method [28]. One ml DPPH solution (0.1 mM) in methanol is blended with 1.0 mL of various concentrations of *U. lactuca* (100–600 µg/mL) extracts. The mixture was kept in the dark for 30 min. Distilled water is taken as the reference standard. One ml DPPH solution and 1 mL methanol were marked as the control. The decrease in absorbance was measured using a UV-visible spectroscopy (UV-Vis) Spectrophotometer at 517 nm. Ascorbic acid was the standard used. The percentage of inhibition was calculated using the following formula: Inhibition %= $(Ab_{blank} - Ab_{test}/Ab_{blank}) \times 100$.

$ABTS^{\bullet+}$ radical cation scavenging activity

The antioxidant capacity was determined by ABTS●+ radical cation scavenging activity following the improved method described

by Re *et al*. [29,30], with slight modification. 2, 2′- Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) ABTS●+ was prepared by reacting with 7.0 mM ABTS stock solution, 2.45 mM potassium persulfate. The mixture was left in the dark to stand at room temperature for 16 h. The ABTS solution was then diluted with 5.0 mM PBS (pH 7.4) to an absorbance of 0.70±0.02 at 730 nm. To 1.0 mL of diluted ABTS●+solution, ethanol and seaweed extracts of varying concentrations of *U. lactuca* (100–600 µg/mL) were added in a series of test tubes. After 10 min, the absorbance was measured at 730 nm. The ABTS●+radical-scavenging activity of the extracts were expressed by: % of Radical inhibition = (Ab control - Ab sample/ Ab control) × 100.

Synthesis of silver nanoparticle and its characterization

Algae-based silver nanoparticle synthesis

Seaweed-based green synthesized silver nanoparticles were prepared by adding 10 mL of *U. lactuca* methanol extract to 90 mL of 0.03 M aqueous silver nitrate ($AgNO_3$) and kept in the dark condition for 48 h to minimize the photoactivation of silver nitrate. Control was maintained without the extract. Visually, a color change from yellowish brown to dark brown in the mixture was observed. After 48 h, the synthesized mixture was analyzed by UV-Vis spectrophotometer and centrifuged at 12000 rpm for 30 min. The pelleted nanoparticles were pooled and oven-dried for 5 h at 60°C to remove the moisture content. The dried silver nanoparticles were powdered and further characterized.

Characterization of synthesized silver nanoparticles

In the UV-visual spectrophotometer, synthesized silver nanoparticles were confirmed by observing the peaks between the range of 200–400 nm [31]. Green synthesized UAgNP was analyzed for the functional groups by Fourier transform infrared spectroscopy (FTIR) (IRSpirit_DESKTOP-U1C7EF1- Instrument1) between 400 cm^{-1} and 4000 cm^{-1} range. Morphological characteristics of the nanoparticles were identified by Scanning Electron Microscopy ZEISS spectra. The crystalline size (D) is determined by the Debye Scherrer equation with X-ray diffraction (XRD) data drawn from an X-ray spectrometer functioned at a current of 30 mA with Cu Kα radiation (λ = 0.1542 nm) and a voltage of 40 kV. EDAX was performed to confirm the presence of elemental silver particles and their concentration. $D=K\lambda/\beta \cos \theta$.

Where D- nanoparticle crystalline size, K- constant (0.98), λ-wavelength 1.54, β-full width at half maximum,

Antimicrobial activity

Microorganisms

Uro-pathogenic microorganisms were procured from the authenticated research institute for the present study. Two bacterial isolates, *E. coli* and *A. baumannii*, and one fungal isolate, *C. albicans*, were employed to assess the antimicrobial activity of *U. lactuca*. Overnight culture in respective media (NA and SDA) with 0.1 OD was used as an inoculum.

MIC by resazurin method

The Minimum Inhibitory Concentration of the extracts (UME, UEA, UHE, UCE,) and Silver nanoparticle was determined by the Resazurin method against two bacterial isolates *E. coli*, *A. baumannii*, and a fungal pathogen *C. albicans* [32,33]. Three sterile 96 well plates were labeled, and 100 µL of the sample was pipetted into the first well of the plates. To all other wells, 50 µL of media (nutrient broth for bacterial culture and potato dextrose broth for Candida) is added and serially diluted. To each well, 10 µL of resazurin indicator solution was added. 10 µL of microbial suspension (0.1 OD) was added to respective plate wells. Each plate was wrapped loosely with cling film to ensure that the culture did not become dehydrated. The plates were incubated at 37°C for 18–24 h. The volume of resorufin produced was proportionate to the viable cells present and was assessed visually by a change in color from purple to pink or colorless. The lowest concentration at which color change occurred is taken as the MIC value [34]. Antibiotic Streptomycin is used as a control for bacteria, and Ketoconazole for fungi.

Anti-cancer activity of UAgNP

The anticancer activity of *Ulva* AgNPs was identified *in vitro* in the HeLa cervical cancer cell line. The cell viability and proliferative potential were determined based on their metabolic activity by MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) assay. The adherent culture medium was replaced with dimethylsulfoxide (DMSO) dissolved medium containing varying concentrations (100 µg/mL to 6.25 µg/mL) of UAgNPs and incubated for 24 h. Sequentially, the cells were washed with PBS buffer and incubated with MTT reagent (1 mg/mL) at 37°C for 30 min. The formed formazan crystal was dissolved in 1 mL of DMSO, and the plates were read spectrophotometrically at a wavelength of 570 nm. The cells were also observed microscopically. The percentage growth inhibition was calculated using the formula below:

$$\% \text{ Cell inhibition} = [(At-Ab)/(Ac-Ab)]\times 100$$

where Ab is the absorbance value of the blank, At is the absorbance value of the test compound, and Ac is the absorbance value of the control [32].

Statistical analysis

The experiments were performed in triplet and data were reported as mean values with standard deviation.

RESULTS AND DISCUSSION

Qualitative phytochemical analysis

The present study braces the evidence of phytochemicals prevailing in the five extracts UAE, UHE, UME, UEAE, and UCE (Table 1). Among the five solvents, Hexane, Methanol, and Ethyl acetate extracts demonstrated the presence of all tested metabolites. Alkaloids, Saponins, and Tannins were absent in aqueous and chloroform extract. Methanol extract with a

Table 1: Phytochemicals present in various solvent extracts of *Ulva lactuca*

Phytochemicals	Aqueous extract (AE)	Hexane extract (HE)	Methanol extract (ME)	Ethyl acetate extract (EAE)	Chloroform extract (CE)
Alkaloids	-	+	++	+	-
Phenols	+	+	+++	+++	+
Flavonoids	+	+	+++	+++	++
Tannins	-	++	++	+++	-
Saponins	-	++	++	+	-
Terpenoids	+	+	+	++	+
Steroids	+	+	++	++	+
Carbohydrates	++	+	++	+	+
Glycosides	++	+	++	+	++
Amino acids	+	++	++	+	+
Proteins	+	+++	++	++	++

+ Present, ++ present in moderately high concentration, +++ present in High concentration, - Absent

Table 2: Quantitative phytochemical analysis of Methanol extract of *Ulva lactuca*

Metabolites/sample	Flavonoids (mg QE/g)	Total phenols (mg GAE/g)	Tannins (mg TAE/g)	Steroids mg/dL
Ulva lactuca	2.56±0.30	3.66±0.15	2.90±0.61	3.40±0.30

Values are expressed as mean±standard deviation (n=3). QE: Quercetin equivalent, GAE: Gallic acid equivalents

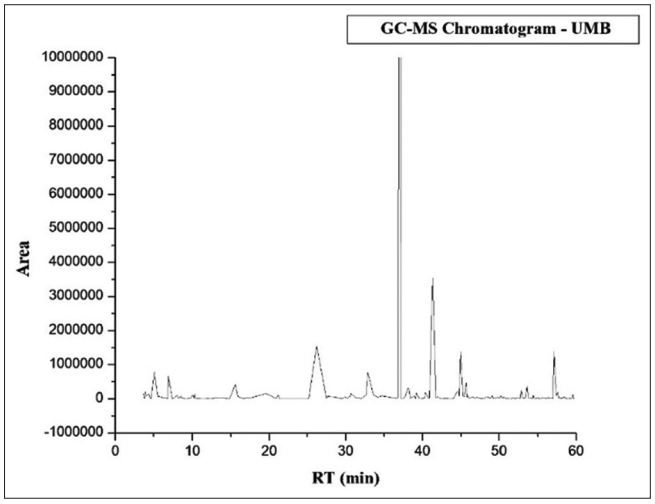


Fig. 2: Gas chromatography-mass spectrophotometer chromatogram of methanol extract of *Ulva lactuca*

higher content of metabolites proves methanol as a choice of solvent for phytochemical extraction [35]. Further analysis was carried out using methanol extract. The phytochemical profile reveals the prospective bioactivity properties of the green seaweed *U. lactuca* [18,27]. The presence of carbohydrates, amino acids, and proteins proves the nutritional value of edible seaweed [36-38].

Quantitative phytochemical analysis

The estimated total contents of Phenol, Flavonoids, Tannins, and steroids in the methanol extract of *U. lactuca* are depicted in Table 2. Total Flavonoids estimated was 2.56±0.30 mg QE per gram sample, TPC 3.66±0.15 mg GAE/g, Tannic acid equivalent tannins 2.90±0.61 mg/g, and 3.40±0.30 mg/dL of steroids. Prasedya reported [39] that phenol compounds supported antiviral, anti-inflammatory, and anticancer properties, whereas flavonoids had antibacterial, antioxidant, and spasmolytic action. Tannins have been found to have antiviral, anticancer, antibacterial, anti-inflammatory, and antioxidant activities, according to Ogawa and Yazaki [40]. The presence of flavonoids, tannins, and phenol components indicates that *U. lactuca* has therapeutic significance.

GC-MS analysis

The GC-MS chromatogram (Fig. 2) revealed prominent peaks of various metabolites with different retention times. The metabolites were identified by comparing the known components in the NIST 14s – lib library. Table 3 illustrates the metabolites' molecular formula, molecular weight, and structure. Bioactivity of metabolites – Dodecanoic acid, Azelaic Acid, n-hexadecanoic acid, Phytol, Valeric acid, Piperidine, Phenol 2 propyl and 1,2 Benzenedicarboxylic acid, di-isooctyl ester, etc., were identified by using Pub chem. According to Islam *et al*., Phytol has anti-inflammatory, antioxidant, and antimicrobial activity [41-43]. Supriya and Haritha reported the antifungal activity of Hexadecanoic acid [44]. Azelaic Acid is a dicarboxylic fatty acid that exhibits anti-bacterial, antifungal, and anti-neoplastic activity [45].

Anti-oxidant activity

IC 50 was determined from the plot plotted corresponding to the % of radical scavenging of DPPH. Ethyl acetate, methanol, and hexane extracts recorded the highest DPPT antioxidant properties with 309.871 µg/mL, 432.264 µg/mL, and 432.273 µg/mL, respectively, whereas in ABTs, aqueous, methanol, and ethyl acetate showed the highest activity at 39.090 µg/mL, 104.43 µg/mL, and 252.491 µg/mL, respectively (Fig. 3).

Characterization of silver nanoparticles

The AgNPs synthesis was performed with 0.03 M of silver nitrate solution with seaweed extract in the ratio of 1:10, respectively, in Erlenmeyer flask. Silver nitrate solution (0.03 M) without extract was maintained as a control. The solution remained colorless and showed no color change even after 48 h. The reduction of silver nitrate ($AgNO_3$) was visually confirmed by the change of color from yellowish-brown to reddish-brown after 30 min of reaction and dark brown after 48 h (Fig. 4).

Das *et al.* [46] reviewed the application of algae-mediated silver nanoparticles.

UV-Vis analysis

The characterization of silver nanoparticles based on surface plasmon resonance vibration observed at 455 nm confirmed the synthesis of AgNPs using green seaweed *U. lactuca* extract (Fig. 5).

FTIR analysis

FTIR analysis was conducted to identify functional groups capped with the green synthesized silver nanoparticle of marine alga *U. lactuca.* Fig. 6 epitomizes the peaks observed at 3356 cm^{-1} representing O-H alcohol, 3379 cm^{-1} for O-H carboxylic acid, and peaks at 3219 cm^{-1}, 2824 cm^{-1} are due to the N-H Amine group. The spectra band at 1435 cm^{-1} indicates O-H bending carboxylic acid, and the peak at 2944 cm^{-1} for C-H aldehyde and C-N Amines at peak 1017 cm^{-1} indicates the various functional groups present in the seaweed-based silver nanoparticle UAgNP [47,48].

Scanning electron microscopy of *U. lactuca* silver nanoparticles

The high-density silver nanoparticles created by processing *U. lactuca* extract are shown in the SEM image (Fig. 7), which further supports the emergence of silver nanostructures. The silver nanoparticles appeared evenly dispersed and had a size range from 16.38 nm to 24.56 nm. They were spherical-shaped and highly distributed with aggregation.

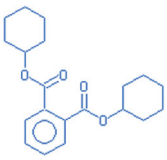
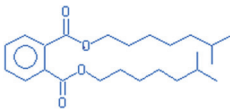
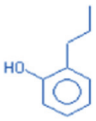
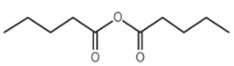
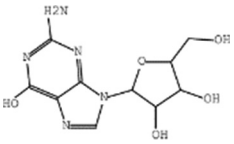
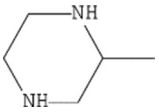
Energy-dispersive X-ray (EDX) spectrum and XRD analysis

An EDX spectrometer determined the presence of elements and atomic proportions of seaweed-mediated AgNPs (Table 4). The analysis through the EDX spectrum recorded a sharp peak at 3 keV. The presence of an elemental silver signal in the silver nanoparticles is confirmed in Fig. 8. The composition of silver in silver nanoparticles is 40.39% wt and 10.29% atom. The XRD pattern (Fig. 9) showed two intense peaks in the spectrum of ∘2ɵ values ranging from 20 to 80. The peaks of 38.45 and 44.48 correspond to (111) (200), planes for silver and indicating crystalline [49].

Antimicrobial activity

The antimicrobial activity of four solvent extracts (UEAB, UCB, UHB, UMB) and green synthesized silver nanoparticles (UNPS) were determined by the resazurin method. Upon incubation, viable cells convert resazurin (purple) into resorufin (Pink or Colorless). The lowest concentration indicated by the colour change from purple to pink or colorless (Fig. 10) was taken as minimal inhibitory concentration (Table 5). Indicates the various concentrations at which the development of resorufin by viable cells. The present investigation shows that all the solvent extracts and nanoparticles have antimicrobial activity at varying concentrations. Among five samples, *Ulva*-based nanoparticles exhibited MIC of 250 µg/mL

Table 3: Gas chromatography-mass spectrophotometer metabolite profile of methanol extract of *Ulva lactuca*

S. No	Structure	Compound name	Retention time (RT)	Molecular weight	Molecular formula	Bioactivity
1.		1,2 Benzenedicarboxylic acid, dicyclohexyl ester	22.35	330.4 g/mol	$C_{20}H_{26}O_4$	-
2.		Azelaic acid	15.08	216.27 g/mol	$C_{11}H_{20}O_4$	Antibacterial
3.		1,2 Benzenedicarboxylic acid, di-isooctyl ester	23.43	390.6g/mol	$C_{35}H_{48}O_8$	Fungicidal
4.		Phenol 2 propyl	12.82	164.24 g/mol	$C_{11}H_{16}O$	Antimicrobial
5.		Dodecanoicacid, 10-oxo-	16.6	214.3 g/mol	$C_{12}H_{22}O_3$	Bactericidal
6.		Pentadecanoic acid	17.22	242.4 g/mol	$C_{15}H_{30}O_2$	-
7.		n-Hexadecanoic acid	18.98	256.42 g/mol	$C_{16}H_{32}O_2$	Anticancer, anti-fungal
8.		Phytol	21.07	296.5 g/mol	$C_{20}H_{40}O$	Anti-inflammatory, anti-oxidant, antimicrobial
9.		Valeric acid (PENTANOIC ACID)	17.74	102.13 g/mol	$C_{10}H_{18}O_3$	Fungicide Flavoring agent
10.		Guanosine	26.192	283 g/mol	$C_{10}H_{13}N_5O_5$	Antidepressant
11.		Piperidine	30.645	100 g/mol	$C_5H_{12}N_2$	Analgesic

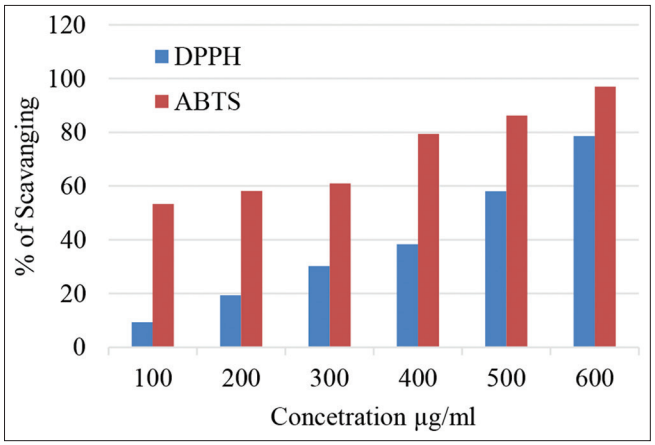


Fig. 3: Antioxidant activity of *Ulva lactuca*

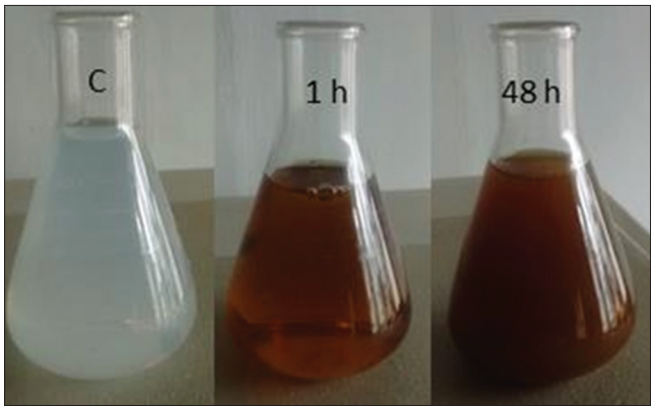


Fig. 4: Green synthesis of silver nanoparticle using *Ulva lactuca* methanol extract

for *E. coli*, except chloroform; other extracts showed 500 µg/mL. *A. baumannii* was inhibited at the concentration of 500 µg/mL with UCB and UHB. All the extracts were able to inhibit *Candida* spp. At the lowest concentration range of 125–250 µg, whereas nanoparticles exhibited MIC of 500 µg [50]. The preliminary antimicrobial (MIC) study exposed the antimicrobial activity of *U. lactuca* extracts and green synthesized silver nanoparticles. Bensy *et al.* [8] stated the antimicrobial activity of *Ulva-based* iron nanoparticles. The present study marks the potential anti-fungal activity of *U. lactuca.* Further expansion of the investigation will design an alternative to antifungal and antibacterial agents with an eco-friendly strategy.

Anticancer activity of *U. lactuca* silver nanoparticle

Green synthesized *U. lactuca* silver nanoparticles were employed by MTT assay to determine anti-proliferative activity on the HeLa cell line. The dose-dependent effect of UNP was observed in the

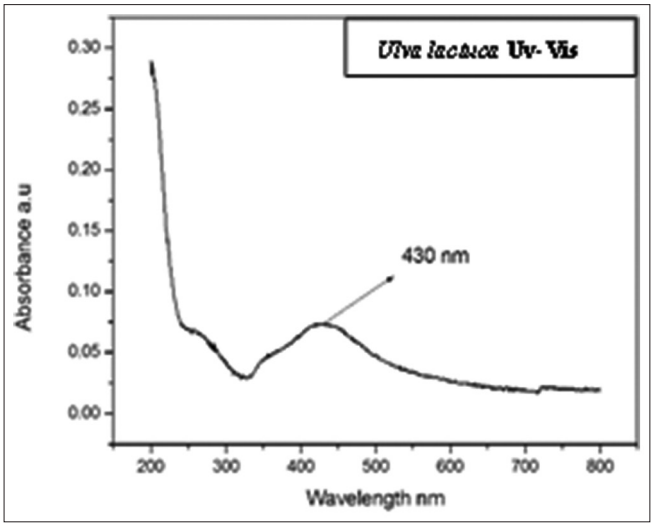


Fig. 5: UV-spectroscopy confirms the formation of silver nanoparticles

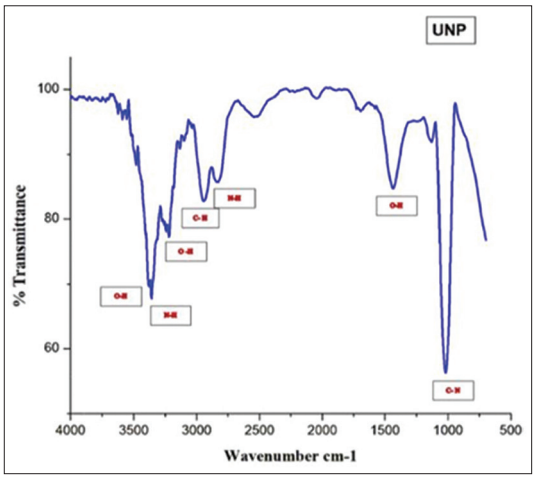


Fig. 6: FT-IR shows the functional groups of Ulva silver nanoparticles

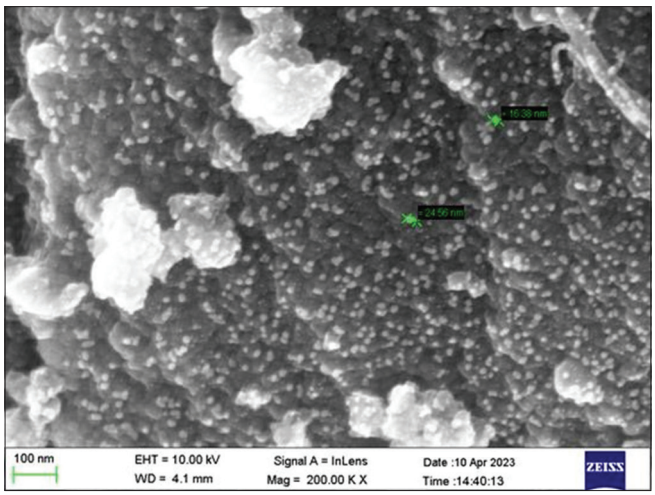


Fig. 7: SEM image of Ulva-based silver nanoparticle

MTT assay. The cell line viability after the addition of various concentrations of 100–6.25 µg/mL of silver nanoparticle was found

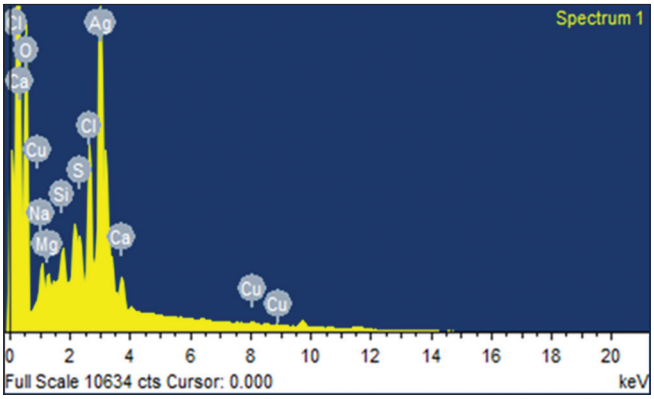


Fig. 8: EDAX

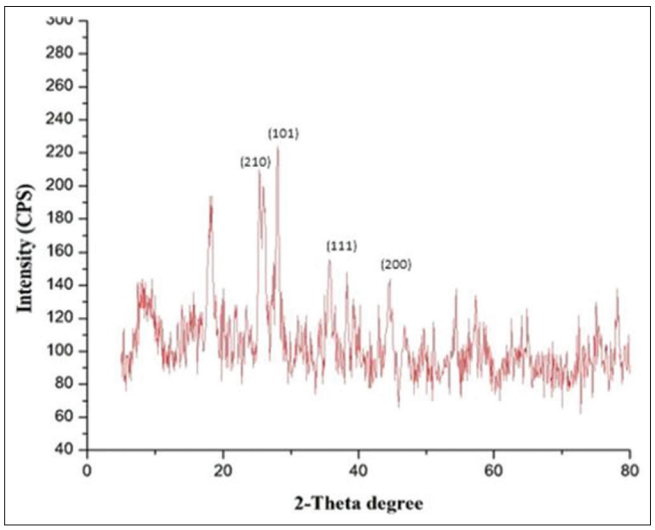


Fig. 9: X-ray diffraction spectrum of Ulva silver nanoparticle

Table 4: The relative weight and atomic proportions of each element present in the silver nanoparticles synthesized

Element	Weight%	Atomic%
O K	44.69	76.77
NaK	2.70	3.22
MgK	0.98	1.11
SiK	1.34	1.31
SK	0.91	0.78
ClK	6.28	4.87
CaK	1.84	1.26
CuK	0.87	0.37
AgL	40.39	10.29
Totals	100.00	

to be 69.1–16.43%. Fig. 11 represents the IC_{50} value of *Ulva-based* silver nanoparticle found to be 30.63 µg/mL. The results (Fig. 12) indicate a dose-dependent cell death in the presence of UAgNPs. Acharya *et al.* [51] reported efficient anticancer activity of *U. lactuca* against colon cancer cell line [52]. Over the past decade, the effectiveness of algae as an anticancer agent has been established, indicating that Seaweeds can be employed as biological agents in treating human diseases, including cancer [53]. The evaluated nanoparticle's mode of action still needs to be investigated. Further studies in edible seaweeds pose a promising alternative to chemical therapy.

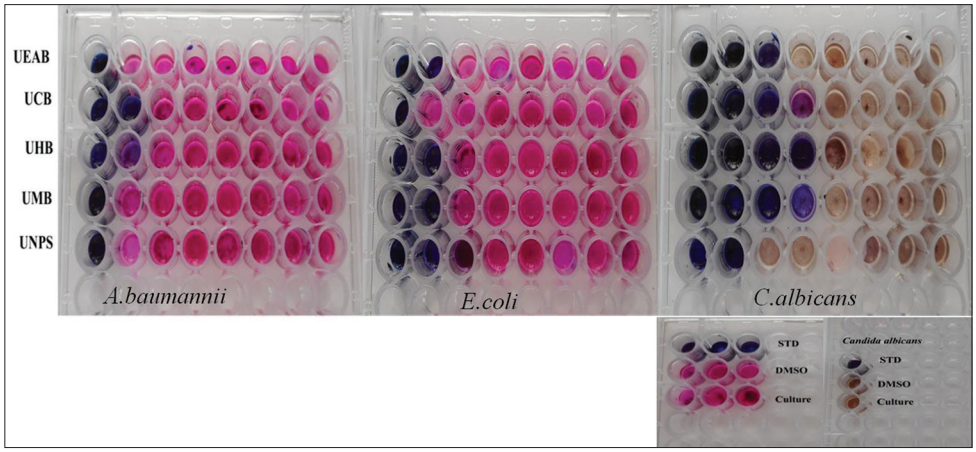


Fig. 10: Resazurin test indicating MIC at various concentrations

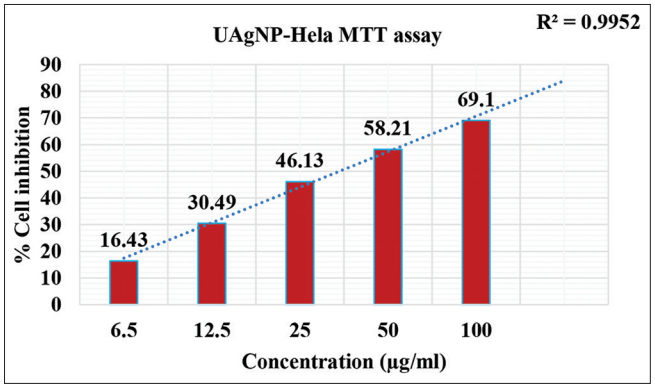


Fig. 11: IC_{50} value of Ulva-based silver nanoparticle

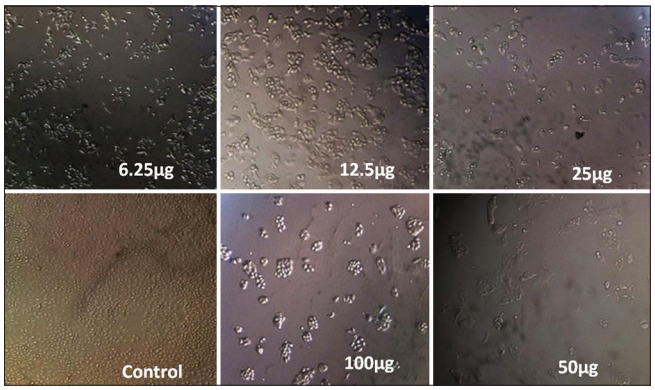


Fig. 12: UAgNP Dose-dependent cell death in HeLa cell line

Table 5: MIC values of various extracts and silver nanoparticles of *Ulva lactuca*

Sample	*Escherichia coli*	*Acinetobacter baumannii*	*Candida albicans*
	MIC µg/mL	MIC µg/mL	MIC µg/mL
UEAB	500	1000	250
UCB	1000	500	125
UHB	500	500	125
UMB	500	1000	125
UNP	250	1000	500

CONCLUSION

Antibiotic resistance among microorganisms can be minimized by careful antibiotic and medication practice. The current scenario demands pharmacognosy, a natural resource-based alternative to control the emergence of resistance. Since ancient times, traditional foods have played a significant role in managing various diseases. Edible Seaweed *U. lactuca* contains a variety of potential phytochemicals with diverse biological functions. The presence of flavonoids and phenol supports the anti-oxidant and antimicrobial action. Environmentally friendly, sustainably produced silver nanoparticles have a wide range of applications in the biomedical industry, including developing antimicrobial drugs. Identifying the metabolites that produce the observed effects in controlling UTI and as a potential drug of choice for cervical cancer may strengthen the medical application of green seaweed.

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AUTHOR'S CONTRIBUTION

Babini C K: Investigation, Methodology, Resources, analysis, and Writing - original draft. Reena A: Conceptualization, Validation, original draft, and Supervision.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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