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Original Article

Characterization and Biofabrication of Silver Nanoparticles Utilizing *Isochrysis* Extract along with its *in vitro* Antibacterial and Antioxidant Applications

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ABSTRACT

Background: At present, bio-green methods are more expedient than physical and chemical approaches due to their prompt, simple, lucrative, scalable and conservational synthesis of nanoparticles. **Aim:** This study investigates the extracellular biogenic synthesis of silver nanoparticles (AgNPs) using the ethanolic-hexane extract of *Isochrysis* sp. (EHEI). **Materials and Methods:** Biosynthesized AgNPs were nano-characterized by UV-Vis spectroscopy, Field Emission Scanning Electron Microscope (FESEM), Dynamic light scattering (DLS), X-ray diffraction (XRD), Energy Dispersive X-ray (EDX), Fourier Transform Infrared Spectra (FTIR), and Gas Chromatography Mass Spectrometry (GCMS) and finally examined for antibacterial and antioxidant activity. **Results:** The biosynthesis of AgNPs was evidenced with UV-Vis spectra showing surface plasmon resonance (SPR) at 421 nm; fabrication of highly stable, well dispersed, spherical AgNPs with an average size of 64.47 nm which were evidenced by FESEM. The crystalline nature of AgNPs was apparent from the diffraction peaks of XRD. The Zeta potential value of-22.3 mV demonstrated the colloidal stability of AgNPs. FTIR spectra showed that functional groups existing in fatty acids, lipids, proteins, xanthophylls, polyphenols and amines are accountable for the formation and stabilization of AgNPs. GCMS confirmed that biomolecules like fatty acid ethyl esters (FAEE) are involved in capping, biochemical reduction of Ag^+ ions and stabilization of AgNPs. Also, the synthesized NPs displayed high antimicrobial activity against pathogenic G+ve and G-ve bacteria. *In vitro* antioxidant properties were ascertained by 1,1-diphenyl-2-picryl-hydrazyl (DPPH), hydrogen peroxide and reducing power assays which established that AgNPs have an incomparable scavenging potential than the extracts. **Conclusion:** Use of such a microalgal system postulates an alternative template for biofabrication of multifunctional nanomaterials in extensive prototype that might be extensively applicable in biomedical sectors.

Keywords: *Isochrysis*, AgNPs, FESEM, GCMS, Antibacterial, Antioxidant.

INTRODUCTION

Oxidative stress and Reactive Oxygen Species (ROS) are commonly renowned to be injurious to our health; they play a part in start/succession of numerous pathologies arraying from cardiovascular ailments to cancer.[1] Antioxidant agents are proficient to impede oxidative stress and alleviate its outcome on human welfare; they also proved to have a virtuous extent of competence in provisions of disease prohibition/therapy and entirely free from essential adversative effects which attained massive interest from the biomedical researchers too.[2] The dearth of reports registered to explore the quantification and documentation of antioxidant compounds of microalgae although more antioxidant summaries in microalgae have been stated.[3]

Nano-Biotechnology has renovated nanomaterial production by conferring a green synthetic framework exploiting biological systems such as microalgae, which have astounding ability to acquire metal ions and fabricate NPs by detoxification method. Nano-biotechnology plays a vital role in evolving as an alternative and effective therapy for treating various diseases.[4] Nanotechnology is defined as the production of materials ranging in size from 1-100 nm scale. Biological method of NP synthesis is a vast developing technique in nanotechnology field.[5] Recently biological materials like algae, bacteria and fungi were used in NP synthesis. Algae are identified as bionanofactories because of

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their live and dead dried biomass can be used for metallic NP synthesis.[6]

Microalgae generate a profuse sum of biomolecules like carotenoids, fatty acids, proteins, polysaccharides, sterols and vitamins with biomedical characteristics, that can be worthily utilized in medical, pharmaceutical and nutraceutical areas.[7] Biosynthesis of metal NPs employing microalgae is a promising domain in nanotechnology and biotechnology. Nano-characterization techniques are used to establish various physical parameters.[8] In current history, there are not many investigations registered that alga being exploited as a biofactory for development of metallic NPs.

Nature is the true source of lead compounds, exploration of largely divergent algae for its natural biomolecules helps in isolating more than 15000 economically important novel elements. In this study, *Isochrysis* sp. a golden brown marine photoautotrophic microalgae of haptophyta class, multiply in harsh conditions, utilize light and inorganic nutrients to synthesize secondary metabolites that are bioactive has been selected and also have superior nutritive qualities.[9] It is enriched in lipids, particularly polyunsaturated fatty acids (EPA and DHA), which are believed to avert circulatory ailments and stimulates cognitive development.[10] It comprises phytochemicals like polysaccharides, fatty acids, carotenoids, vitamins, and sterols which have effective cardiovascular agent, antibacterial, antioxidant, hypocholesterolemic, anti-inflammatory, immunomodulatory, and cytotoxic properties. Several papers have been undertaken on the extraction of *Isochrysis galbana* polysaccharides,[11] but quite a few reports evidenced for the synthesis of microalgae metal NPs with biomedical properties.[12] This paper endorses a green methodology for biological AgNP synthesis exercising the *Isochrysis* sp. crude extract following characterization and biomedical studies. The successfully biosynthesized AgNPs presented good antioxidant and antibacterial effects.

MATERIALS AND METHODS

Preparation of Extract

Freeze-dried microalgae (5 g) were extracted with 500 mL of solvents ethanol:hexane (7:3), hexane and acetone (Figure 1), for 20 min at 40°C with rotational velocity 6000 rpm using an Ultra-Turrax T-25 Homogenizer. The resultant slurry was cooled, centrifuged at 3000 rpm for 15 min before being filtered. The filter cake was re-extracted for 20 times until it became colorless. The filtrates were amalgamated; concentrated in a rotary vacuum evaporator at 30°C–45°C. All processes were carried out in the dark; extraction conditions were given in Table 1. Later extracts were lyophilized, and the quantity of substances extracted was expressed as percentage by weight. The freeze-dried powder was deemed as the *Isochrysis* sp. crude extract.[13] The crude extract was evaporated for Ethanol-hexane Extract *Isochrysis* (EHEI), Hexane Extract *Isochrysis* (HEI) and Acetone extract *Isochrysis* (AEI) resulting in a concentrated thick residue.

Phytochemical and Biochemical Screening

The powdered extracts were utilized for phytochemical tests with little modifications.[14] TCC for all extracts were examined as per the Lichtenthaler HK protocol, 1987, by appraising the absorbance at 470 nm for carotenoids.[15] The EHEI, HEI and AEI were assessed for biochemical elements such as phenols and total antioxidant activities using gallic acid and AsA as standard correspondingly. For all the test solutions, the TPC of crude extracts were measured at 720 nm (Gallic acid equivalent/g),[16] and TAA were measured at 695 nm (number of equivalents of AsA).[17]

FTIR and GC-MS

FTIR spectra were ensued for EHEI; resolution 4 cm^{-1} transmission mode range-4000 and 400 cm^{-1} Shimadzu IR spectrophotometer, model 840, Japan. GCMS analysis of EHEI was run (Shimadzu/QP2020GC instrument-MS-5975 inert MSD with triple-axis mass selective ion detector) and the account of phytochemical elements was attained.[18]

Preparation of Aqueous Extract of *Isochrysis* sp.

Mix 1 g EHEI powder with 100 mL deionized water, maintained in a water bath (60°C) for 10 min before being filtered through Whatman filter paper (Grade 1) and stored at 4°C for further studies.[19]

Biosynthesis of AgNPs

In a sterile conical flask, aqueous extract (10 mL) and 1 mM aqueous $AgNO_3$ solution (90 mL) were coupled for biosynthesis. In addition, 1 mM aqueous $AgNO_3$ solution (100 mL) was set aside as control. Incubate the mixtures (7 days at 60°C) and the particle formation increases as the temperature augmented to 80°C. AgNPs generation was viewed by a visual colour shift (light green to dark brown) by reducing Ag^+ to Ag^0.[19] Thereafter, the solution was centrifuged at 16,000 rpm (25 min at 4°C), the supernatant was detached to expel unreacted Ag^+ ions. After that, the dark brown pellet that remained beneath the tube was isolated and cleansed from the mixture by rinsing it with sterile distilled water before being lyophilized (LY00555 LYODEL Freeze dryer). For further research, the AgNPs were kept at 4°C.

Nano-characterization of biosynthesized AgNPs

The bioreduction of Ag^+ ions in EHEI was measured by UV-visible spectrophotometer (Shimandzu model SL-210) at the wavelength of 200–700 nm.[20] For DLS valuation, the particle size and zeta potential were conducted at 37°C (Zetasizer-Horiba Scientific NanoPartica Nanoparticle Analyzer SZ-100).[20] The XRD analysis was run at a voltage of 50 kV (Bruker Kappa APEXII instrument) and a current (40 mA) with Cu-Kα radiations (λ =

Table 1: Solvents used in extraction process.

Microalgae	Solvents used	No. of extraction cycles
Dried *Isochrysis* sp. microalgal powder (5g)	Ethanol:Hexane (7:3) (25 mL and 20 min for each cycle)	20
	Hexane (25 mL and 20 min for each cycle)	20
	Acetone (25 mL and 20 min for each cycle)	20

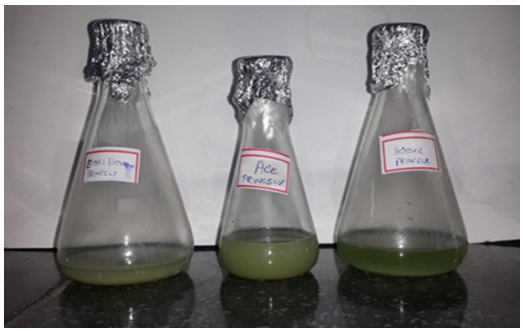

Figure 1: *Isochrysis* extracts from Ethanol:hexane, hexane and Acetone.

1.54060A) in 2θ range (30–80°C).[20] The functional group reliable for the bioreduction of AgNPs was investigated using FTIR. The FTIR data were documented at room resolution (Shimadzu IR spectrophotometer, model 840, Japan) in the mid-infrared region (4000–400 cm^{-1}). The surface of AgNPs were scanned by FESEM (BRUKER INDIA, FESEM-provided with an EDX fitment). A sample of thin film was made with aluminium foil by dwindling a slight volume of sample on top of the copper grid.[21] The elemental composition was ascertained by EDX analysis.[21]

Antimicrobial Activity-Well Diffusion Assay

The well diffusion assay was used to test the antimicrobial efficiency of biosynthesized AgNPs. Gram negative (*P. aeruginosa* and *E. coli*) and Gram positive (*S. aureus* and *B. subtilis*) microorganisms were procured by the GSL Institute of Medical Science in Rajahmundry, India and cultured using Mueller-Hinton (MH) broth. The strains were swabbed on the MH agar plate along with formation of 6 mm diameter wells. The concentrations of 10, 20 and 30 µL of the freshly prepared AgNPs (1 mg mL^{-1}) were put into the appropriate wells; incubated at 37°C. On each agar plate, standard-cefotaxamine (1 mg mL^{-1}) employed; inhibition zone quantified-EHEI and AgNPs.[22] The experiment was effected in triplicates.

Antioxidant Studies

DPPH assay

DPPH assay was analyzed for EHEI and AgNPs.[23] Different concentrations of samples (20-100 µg/mL); AsA – standard used; % inhibition calculated

$$\% \text{ inhibition} = [(A_0\text{-}A_1)/A_0] \times 100$$

Where A_0-control absorbance; A_1-sample absorbance

Hydrogen Peroxide Assay (HPA)

HPA of EHEI and AgNPs was performed.[24] Different concentrations of samples (20-100 µg/mL); AsA–standard used.

$$\% \text{ scavenging activity} = [(A_0\text{-}A_1)/A_0] \times 100$$

Where A_0-control absorbance; A_1-sample absorbance

Ferric reducing power assay (FRAP)

FRAP was analyzed for EHEI and AgNPs.[25] Different concentrations (20-100 µg/mL) of samples were employed with AsA acting as standard. FRAP was computed using the absorbance value. For all of the antioxidant assays, IC_{50} values were obtained using a linear regression curve.

Statistical Analysis

For linear regression, the data were assessed using GraphPad prism (Windows version 7.04). The results of the statistical analysis were worked out using one way ANOVA and SPSS version16 software (SPSS Inc., Chicago, IL). Each experiment's values were depicted as mean ± S.D. (three replicates). The results were meant to be statistically significant (if $p < 0.05$).

RESULTS AND DISCUSSION

Characterization of Extract

The *Isochrysis* biomass dry weight was ascertained to be 5 g. The % yield extractions of ethanol:hexane (1.8 g), hexane (0.5 g), and acetone (0.7 g) were determined to be 36.83%, 10.32%, and 14.71% w/w correspondingly, where ethanol:hexane gave the highest.

Phytochemical screening

In the present study, phytochemical screening for EHEI, EEI, and AEI was performed (Table 2) and concordant with former work.

Total Carotenoid Content (TCC)

Carotenoid content of EHEI, EEI, and AEI extracts were shown in Table 3. It indicated that the highest carotenoid levels were recorded in EHEI (7.83 ± 0.09 mg/g), whereas minimum in HEI (5.91 ± 0.03 mg/g). Similar results showed that the ethanol/water extracts (7.8 mg g^{-1} biomass) of *Isochrysis* sp., *Chlorella*

Table 2: Phytochemical screening for EHEI, EEI, and AEI.

Sl. No	Phytochemicals	EHEI	HEI	AEI
1	Tannin	++	++	++
2	Saponin	++	++	++
3	Flavonoids	++	+	++
4	Steroids	++	+	+
5	Terpenoids	+	+	+
6	Triterpenoids	+	+	+
7	Alkaloids	++	+	+
8	Anthroquinone	+	+	+
9	Polyphenol	++	++	++
10	Glycoside	+	+	+
11	Coumarins	+	+	+
12	Emodins	-	-	-
13	Anthocyanins	-	-	-

"+" indicates presence of the compounds; "-" indicates absence of the compounds, "++" indicates the high concentration

Table 3: Biochemical contents of EHEI, EEI, and AEI.

Biochemical content	EHEI	HEI	AEI
TPC (mg GAE/g)	27.61 ± 1.8	13.22 ± 1.5	18.31 ± 1.7
TCC (mg/g)	7.83 ± 0.09	5.91 ± 0.03	6.32 ± 0.13
TAA (mg/g)	2.31 ± 0.3	1.45 ± ± 0.7	1.93 ± 0.5

Values were recorded as mean ± SD of three parallel measurements. TPC: Total phenolic content (mg GAE/g extract), TCC: Total carotenoid content as per Lichtenthaler equations (mg/g extract), TAA: Total Antioxidant activity (mg/g AsA equivalent), SD: Standard deviation.

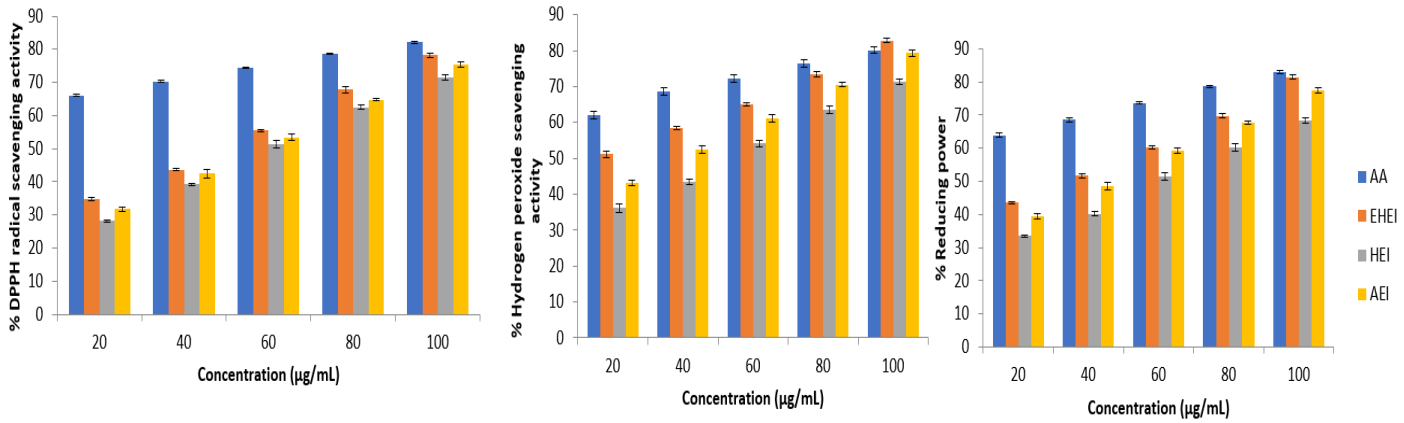

Figure 2: Antioxidant assays of *Isochrysis* extract (a) DPPH, (b) Hydrogen peroxide assay (c) Reducing power assay. All the values are expressed as mean ± SEM, *n*=3.

sp., *T. suecica, Phaeodactylum* sp. had maximum carotenoid concentration.[26] The antioxidant assays (DPPH, HPA and FRAP) demonstrated that carotenoid concentrations had a significant impact.

Total Phenolic Content (TPC)

TPC of EHEI, EEI, and AEI were ascertained, and the results were given in Table 3. The highest TPC was presented in EHEI (27.61 ± 1.8 mg GAE/g) though the lowest was noticed in HEI (13.22 ± 1.5 mg GAE/g). In our study, TPC was found to be higher in EHEI which might be attributable to polarity variances in the solvents used. Past report disclosed methanol extract of *Chlorella marina* had superior activity preceded by diethyl ether and hexane extract.[27]

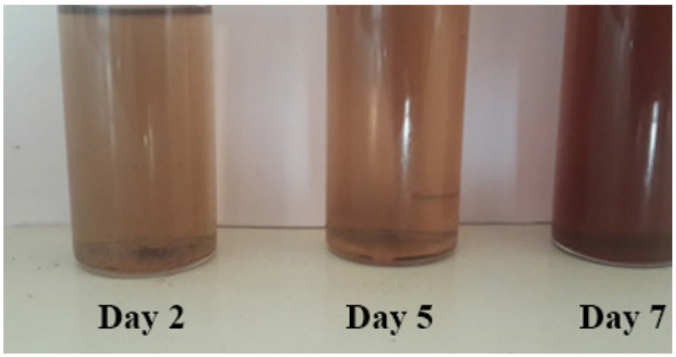

Figure 3: Biofabrication of AgNPs using EHEI.

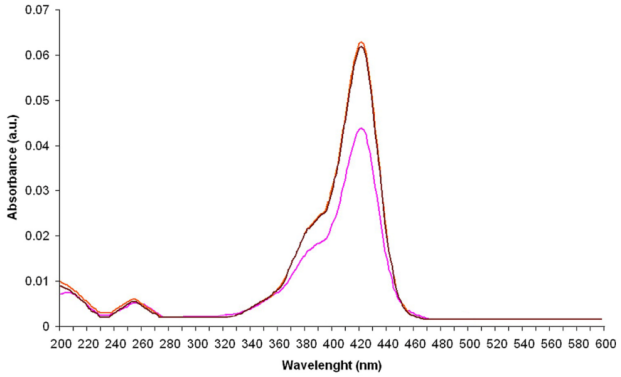

Figure 4: UV-Vis-Spectra of AgNPs using EHEI.

Total Antioxidant Activity (TAA)

TAA of EHEI, EEI, and AEI were showed in Table 3. The highest TAA was noted in EHEI (2.31 ± 0.3 mg/g AsA equivalent) whereas least activity was viewed in the HEI (1.45 ± 0.7 mg/g AsA equivalent). Earlier studies stated that methanolic extracts (*Desmococcus olivaceous* and *Chlorococcum humicola*) of green microalgae established stronger antioxidant potential as compared to ethanolic and acetone extracts.[28] The ranking order for TCC, TPC and TAA was as follows EHEI > AEI > HEI. The EHEI showing high polyphenolic and carotenoid contents were directed to FTIR and GCMS analysis to identify the active biochemicals present. EHEI powder obtained was kept at 4°C for further work.

Antioxidant assays

All the extracts preserved scavenging abilities at various degrees. The scavenging influence was excellent for EHEI extracts compared to the HEI and AEI extracts. The IC_{50} values for AsA was 5.59, 8.14 and 8.09 µg/mL; EHEI extract was found to be 42.55, 21.84 and 31.33 µg/mL; HEI extract was 52.53, 44.74, and 51.63; AEI extract was 46.65, 31.04 and 36.22 for DPPH (Figure 2a), HPA (Figure 2b) and FRAP (Figure 2c) assays indicate ethanol:hexane as the suitable solvent for extracting phytochemicals from *Isochrysis*. The antioxidant potential ranking order was as follows: AsA > EHEI > AEI > HEI. Past research highlighted the remarkable characteristics showcasing the enhanced efficiency of free radicals scavenging in 80% methanol extracts of microalgae.[29] Previous reports suggest that *D. olivaceous* methanol extract (39%) and *C. humicola* acetone extract (15%) had scavenging activities congruently.[28]

The outcomes imply that whilst reviewing microalgae as a reservoir of natural antioxidants, not just carotenoids but also fatty acids, lipids, proteins, xanthophylls, polyphenols and amines must be addressed. Besides, it should be emphasized that biochemicals and carotenoid in some microalgae can be boosted by manipulating environmental conditions.[30] Moreover, the antioxidant properties of microalgae extracts were apparently caused by phenolic constituents.[26] However, both carotenoid and TPC have conferred to its antioxidant effects in the current study. Moreover, grounded on the results attained the antioxidant properties of microalgae shows that it can be used for a variety of infectious diseases.

Characterization of EHEI-AgNPs

The biogenic synthesis of AgNPs exercising EHEI was lucratively established when incubated with 1mM $AgNO_3$ (Figure 3). Upon addition of Ag^+ ions in the light, the samples transformed color (pale yellow to dark brown) with strength mounting during the period of incubation. The emergence of dark brown color was a clear signal for AgNPs fabrication in the reaction mixture.

Besides, seaweeds were exploited as bio-reductant for the Ag salt reduction during the formation of AgNPs.[31] The dark brown color viewed confirms the development of AgNPs and it was evident with UV-vis-spectra. The surface plasmon resonance absorption band triggered by free electrons that was liberated by metal NPs during the redox reaction. Figure 4 depicts the spectrum of UV-vis registered in the peak position at 421 nm regions for AgNPs. The UV-vis-spectra were detailed for the extract along with 1 mM $AgNO_3$ solution at varied time periods (0, 1, 2, 3, 4, 5, 6 and 7 days). The formation of silver oxide (Ag_2O) was the primary reaction which was further reduced into AgNPs. The colour changes from light green to brown and turns darker with upsurge in the incubation period and lastly reddish brown attained after ten days due to the excitation of SPR that establishes the AgNPs generation with exclusive optical properties.[32] The change in colour was proportional to the EHEI concentration and temperature, which could be attributable to the presence of reducing agents like fatty acids, lipids, proteins, polyphenols, xanthophylls, and amines. The OH-group in polyphenols reacted with Ag^+ to generate unstable AgOH, which in turn was further oxidized to Ag_2O and subsequently reduced to AgNPs. The broad plasmon band monitored depicts polydispersed particles as a result of its size distribution.

FESEM designate the shape of the NPs were spherical and hexagonal with some aggregated and irregular particles. AgNPs were polydipersed, as observed under FESEM (Figure 5). Capping ligands may possibly be a carboxyl groups of fatty acids,

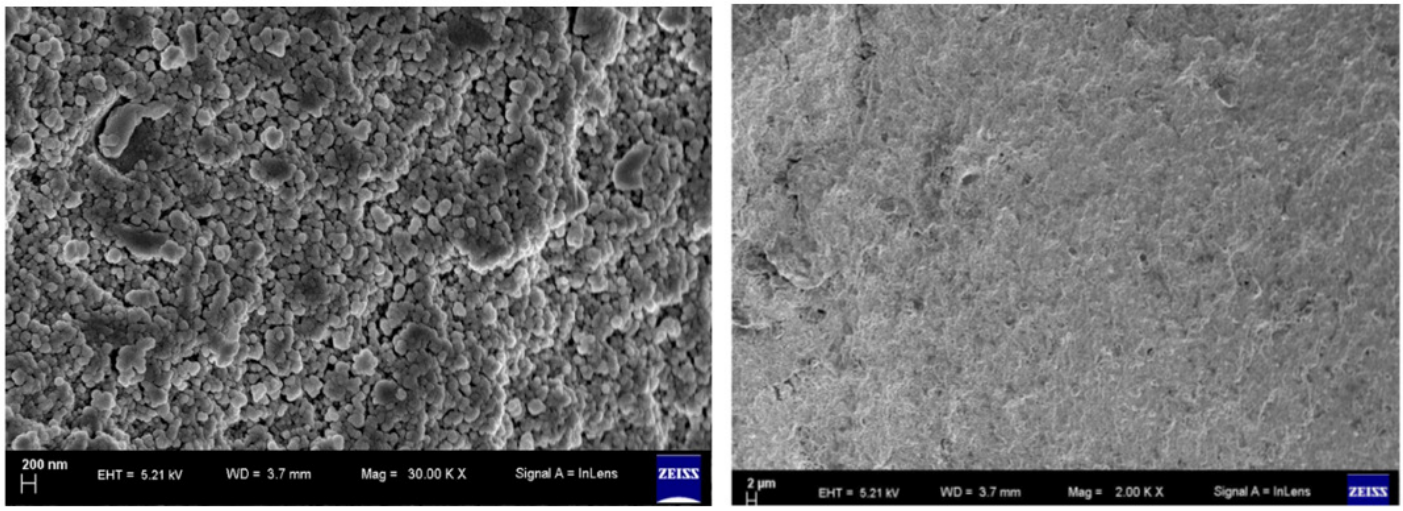

Figure 5: FESEM images of AgNPs using EHEI.

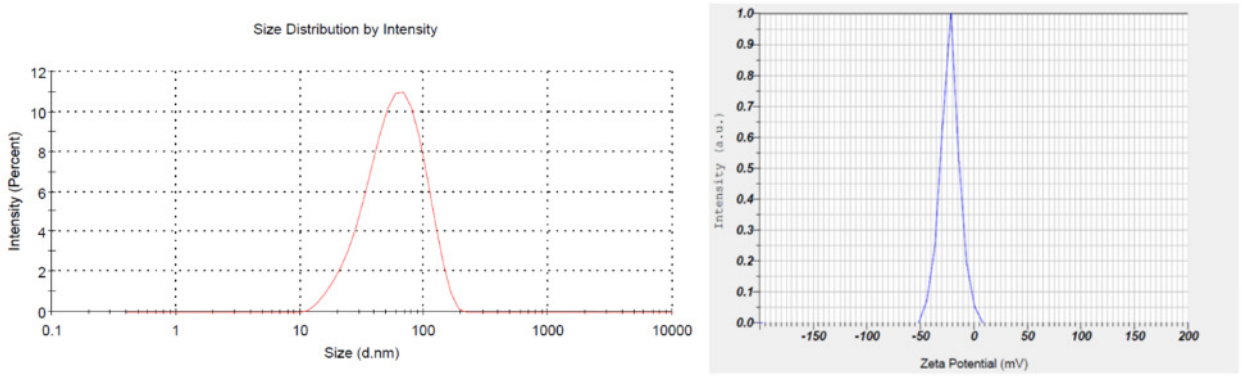

Figure 6: DLS (a) and Zeta potential (b) analysis of AgNPs using EHEI.

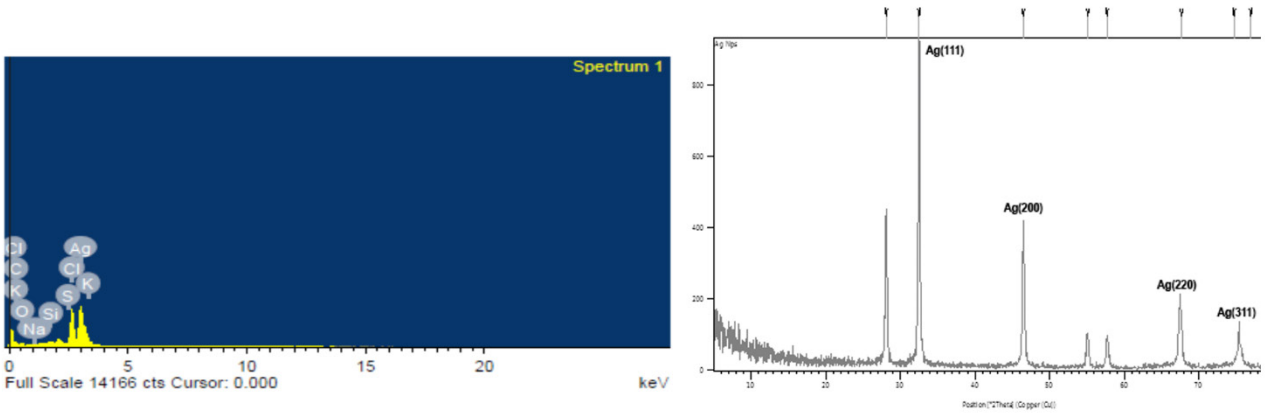

Figure 7: EDX (a) and XRD (b) analysis of AgNPs using EHEI.

terpenoids of xanthophylls, carbonyl and amide linkage of lipids and proteins, carboxyl and hydroxyl groups of polyphenols or amines. Agglomeration of nanoparticles were prevented through bio-capping molecules. Former reports stated, green microalgae were tried for SNP synthesis–*Chlorella vulgaris* (absorbance peak-420 nm; spherical shape, 50-70 nm) and *Chaetoceros calcitrans* (absorbance peak-436 nm; spherical shape, 30-35 nm).[33] Analogous reports attained in the AgNPs biosynthesis from normal and microwave irradiated mixed marine microalgae comprising *I. galbana* by exerting $AgNO_3$ as supplement with medium and its size was 53-79 nm.[34]

The DLS was exerted to ascertain the particle size and its distribution which notifies maximum intensity at average 64.47 nm with size range from 14.27-312.4 nm (Figure 6a). The assessed size was the hydrodynamic dimension of the hypothetical area that scatters with an equivalent pace as of the assessed NPs, as determined by DLS. Consequently, the size ascertained in DLS method was generally larger in contradict with FESEM analysis and evidenced by other scientists too. The polydispersity index was determined to be 0.206 that demonstrates the synthesized NPs were polydispersed.

A negative surface charge of zeta potential-22.3 mV authenticates standard surface charge, high energy interference, and aversion amidst particles that attests the moderate colloidal stability of the particles (Figure 6b). This value infers prevalent electrical charge on the particle surface, that could generate intense repellent force amongst particles to regulate agglomeration.[35]

From EDAX test, strong peaks indicate the reduction of Ag (sharp peaks) and weak peaks of O, C, K, Na, S, S and Cl also adhered to the surface of NPs (Figure 7a). The existence of other elements directs the prevalence of other biochemicals in this final product. Former reports for optimization and characterization of AgNPs employing *I. galbana* demonstrated this desired metal phase.[36]

From XRD pattern, the incidence of Bragg's reflections rises owing to (111), (200), (220) and (321) planes and accords well with those stated for face-centered cubic (fcc) lattice structure of Ag that apparently infers the crystalline nature of AgNPs,[37] (Figure 7b). The peaks corresponding to (111) and (200) were more intense and oriented prevalently. For EDX analysis, besides Ag other signals which were weak specified the incidence of carbon, oxygen, hydrogen, nitrogen and phosphorous elements, that implies the ratification of extracellular phytochemicals layered on top of the metallic surface of NPs aligned adequate with the past literature.[38]

FTIR analysis

FTIR was investigated to spot the promising biochemicals liable for Ag^+ ions reduction and capping of AgNPs. Divergent functional groups implicated in the metal NPs were characterized through FTIR spectra. The FTIR spectrum acquired for both the biosynthesized AgNPs (Figure 8a) and EHEI extract (Figure 8b).

For EHEI extract, the bands were obtained at 879, 1044, 1451, 1662, 2884, 2973, and 3815 cm^{-1}. At 879 cm^{-1}, an intense band found indicates a C=C bending alkene group; at 1044 cm^{-1} a fervent peak spotted illustrate stretching of C-O bond in esters

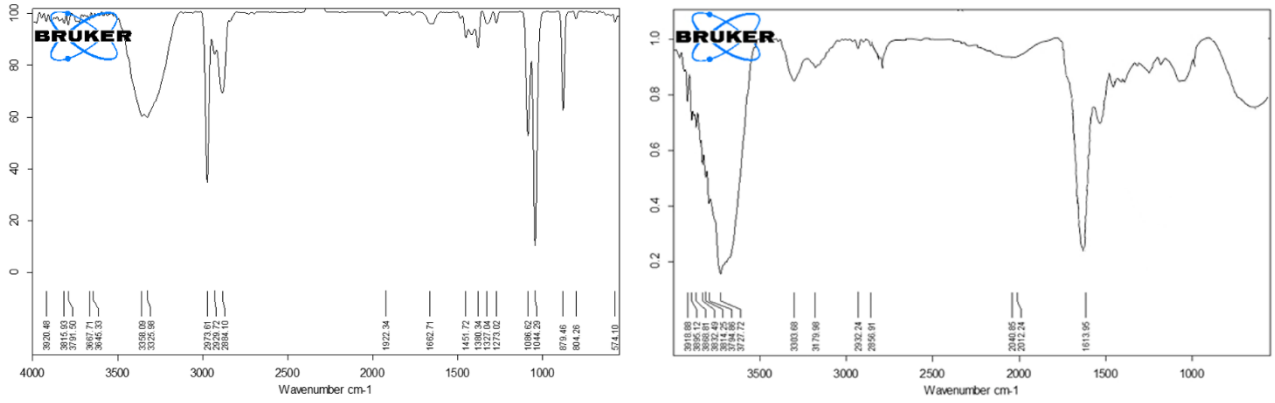

Figure 8: FTIR of EHEI and AgNPs–EHEI.

Table 4: Chemical constituents of EHEI extract.

Peak	Constituents	RT	Area %	Identification	M.F	M.W
1	Ethyl palmitate	22.98	46.01	MS	$C_{18}H_{36}O_2$	284
2	Ethyl oleate	24.56	9.38	MS	$C_{20}H_{38}O_2$	310.28
3	Ethyl stearate	24.73	22.08	MS	$C_{20}H_{40}O_2$	312.30
4	6-(2′-pyridyl)-2-methyl-4-(4′-chlorophenyl) pyridine-3-carboxamide	25.44	22.23		$C_{18}H_{14}ClN_3O$	323.77

and at 1662 cm^{-1} the peak ascribed to strong C=O stretching for Amide I band; a sharp absorbance band at 3815 cm^{-1} implies distorting vibration of strong O-H bond stretching in xanthonoids, flavonoids, and phenolic bio-compounds and N-H stretching of 1° and 2° amines and amides. Likewise, C-H bending alkane, N-H stretching amine, and C-H stretching alkane, were seen at 1451, 2973, and 2884 cm^{-1}, correspondingly. This approves the predominance of a carboxyl groups of fatty acids, carboxyl and hydroxyl groups of polyphenols, carbonyl and amide linkage of lipids and proteins, terpenoids of xanthophylls and amines.

In biofabricated AgNPs, a shift in the band takes place from 2884 to 2856 cm^{-1} when matched to that of EHEI extract. Also, the absorption pattern showed the carbonyl stretching frequency at 1613 cm^{-1}; this shifting frequency from higher (in extract) to lower value (in AgNPs) was accredited due to the reduction of Ag^{+} ions by the biological compounds present in algal extract. The observed difference in the absorption frequencies indicated the involvement of carboxyl, carbonyl and amide linkage, terpenoids, polyphenols, carboxyl and hydroxyl, and amine groups for bio-reduction to AgNPs. These functional groups act as a capping and stabilizing agent during AgNPs formation. Prior results suggested that the stabilisation of AgNPs was also attainable as cysteine residues or free amine groups in the proteins attach with NPs though the electrostatic captivation of negatively charged carboxylate groups.[39]

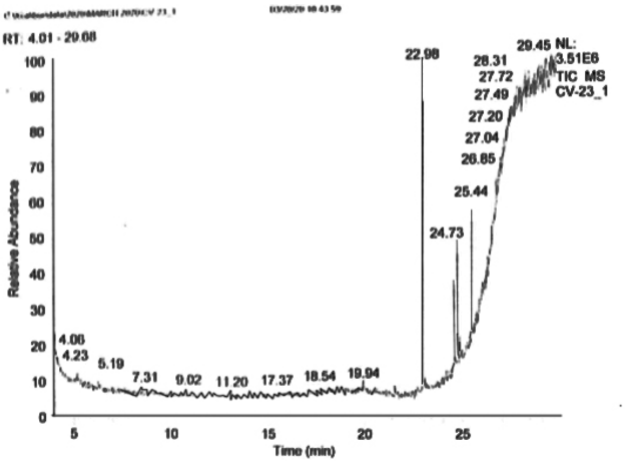

Figure 9: GCMS of EHEI.

GCMS analysis of EHEI

The prevalent elements of the EHEI extracts were Ethyl palmitate (46.01%) pursued by 6-(2′-pyridyl)-2-methyl-4-(4′-chlorophenyl) pyridine-3-carboxamide (22.23%), Ethyl stearate (22.08%) and Ethyl oleate (9.38%) (Figure 9). This analysis concludes that the Fatty acid ethyl esters (FAEE) like ethyl palmitate, ethyl oleate and ethyl stearate majorly imparted in EHEI were liable for bioreduction and stabilization of AgNPs (Table 4).

Antimicrobial Activity

Antimicrobial activity of EHEI and EHEI-AgNPs was explored versus four pathogenic clinical microorganisms accommodating *E. coli, P. aeruginosa, S. aureus* and *B. subtilis*. The findings stated broad antibacterial effectiveness that differed in inhibition versus pathogens. The inhibition diameter of zone was recorded for all wells packed with EHEI (Figure 10a) and AgNPs as depicted (Figure 10b). The activity was documented to be concentration dependent, *i.e* the inhibition zone diameter intensified with increase of concentration (5, 10 and 20 µL) which exemplifies dose-dependent action. The highest inhibition zone was attained versus *E. coli* (9.18 mm for EHEI and 15.61 mm for AgNPs) and *B. subtilis* (7.96 mm for EHEI and 13.26 mm for AgNPs). The minimum inhibition zone was found against *P. aeruginosa* (5.41 mm for EHEI and 10.25 mm for AgNPs) and *S. aureus* (6.33 mm for EHEI and 11.13 mm for AgNPs).

Results show that Gram negative bacteria were more sensitive than Gram positive bacteria. This discrepancy in activity could be attributed to the organism's propensity in this work. The NPs are bound to the plasma membrane and invade the bacterial cells. When AgNPs impale a cell, they form a low-molecular-weight complex in center of the cell, which preferentially damage the respiratory chain cell division resulting in cell death.[40] Most papers proposed that AgNPs may be anchored to the membrane surface,

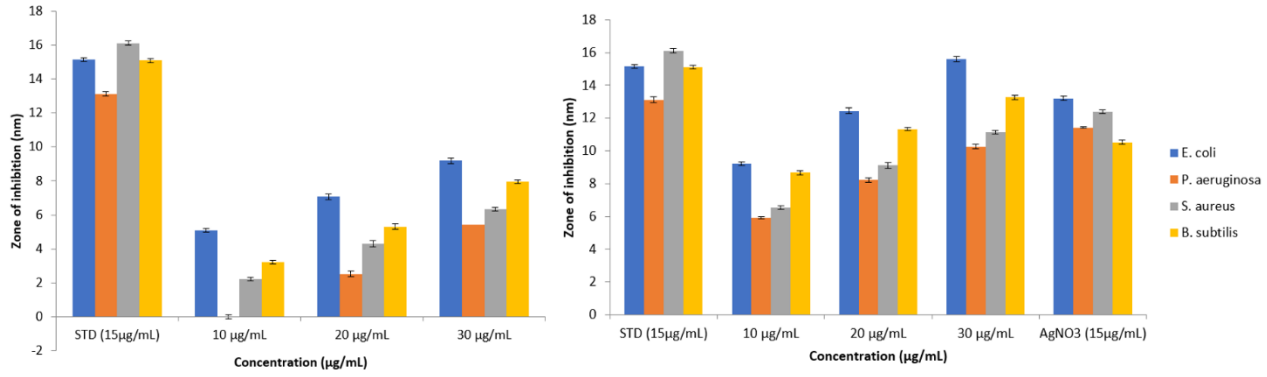

Figure 10: Antimicrobial of EHEI (a) and AgNPs–EHEI (b).

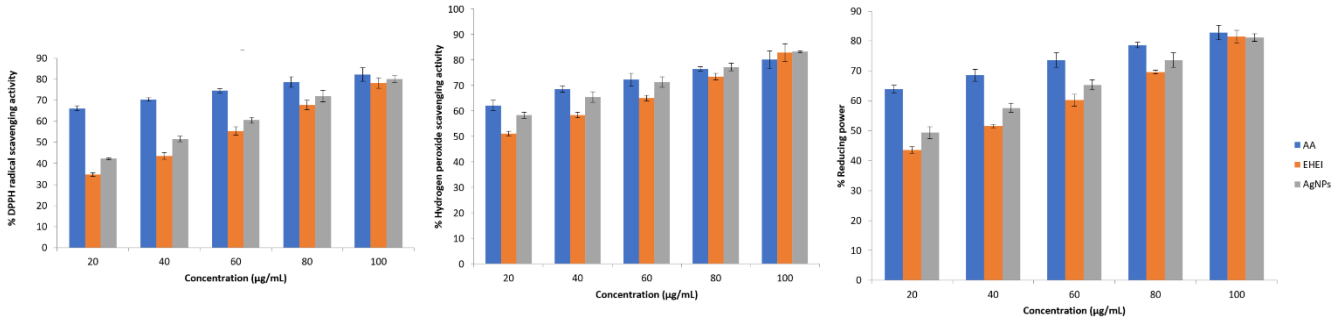

Figure 11: Antioxidant assays of EHEI and AgNPs–EHEI (a) DPPH, (b) Hydrogen peroxide assay (c) Reducing power assay. All the values are expressed as mean ± SEM, *n*=3.

respiration functions of the cell and impairing cell permeability. Past studies, disclosed that acetone extract of *I. galbana* was active versus *S. aureus* (10 mm) and *Micrococcus* sp. (15 mm).[41,42]

Antioxidant Activity

The antioxidant potentiality was measured to Figure out in-case there was any fluctuation in the scavenging properties of EHEI after NP production. The DPPH (Figure 11a), hydrogen peroxide (Figure 11b) and reducing power (Figure 11c) assays were directed to spot the existence of capped anitoxidants in the AgNPs and its proficiency was equated using AsA as standard. These assays augmented in dose dependent manner for both AgNPs and EHEI and extensive efficacy was obvious for AgNPs when equated to extract. The DPPH, HPA and FRAP IC_{50} values of AsA was 5.59, 8.14 and 8.09 µg/mL; AgNPs was revealed to be 31.94, 13.53 and 23.37 µg/mL, compatibly; while for EHEI extract it was noticed to be 42.55, 21.84 and 31.33 µg/mL compatibly. The antioxidant score: AsA>AgNPs-EHEI>EHEI. Previous studies revealed good antioxidant activities due to the biochemicals present in the *Isochrysis* sp.[43,44]

CONCLUSION

It was inferred that the prompt biofabrication of AgNPs by marine microalgae affords a simple, inexpensive alternate paradigm for the nanomaterial biosynthesis in industrial-scale approach which may well be wielded remarkably with tunable biomedical qualities controlled by particle size. The present work documented that AgNPs biosynthesis exerting *Isochrysis* sp. display adequate physical constraints and biomedical outcomes that possibly will act as a convincing nano-drug in biomedical applications. Also, biosynthesised AgNPs could impede bacterial growth and affirmed antioxidant potentialities, which evidences as an substitute for the encroachment of new antimicrobial and anitioxidant agents. In conjunction with new technological advancements, microalgae-derived NPs can be synthesised uniformly and qualitatively, which will optimize their features and functionalities for its commercial usage. So, scale-up and optimisation of biogenic AgNPs would be helpful to explore further that this microalgae might become an massive source in nanobiotechnoogy appliances.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ABBREVIATIONS

ROS: Reactive oxygen species; **AgNPs:** Silver nanoparticles; **EHEI:** Ethanol hexane extract of *Isochrysis* sp.; **HEI:** Hexane extract *Isochrysis*; **AEI:** Acetone extract *Isochrysis*; **TCC:** Total carotenoid content; **TPC:** Total phenolic content; **TAA:** Total antioxidant activity; **FTIR:** Fourier Transform Infrared Spectra; **GCMS:** Gas Chromatography Mass Spectrometry analysis; **FESEM:** Field emission scanning electron microscope; **DLS:** Dynamic light scattering; **XRD:** X-ray diffraction; **EDAX:** Energy dispersive X-ray; **DPPH:** 1,1-diphenyl-2-picryl-hydrazyl; **AsA:** Ascorbic acid.

SUMMARY

Isochrysis sp., a marine microalgae rich in biochemicals, was used in this investigation. EHEI in analogy to other extracts (HEI and AEI), presented valuable biochemical content and antioxidant properties.

Fatty Acid Ethyl Esters (FAEE) were found to be responsible for the superior antimicrobial/antioxidant properties, as evidenced by FTIR and GCMS. The biogenic synthesis of AgNPs was successful employing EHEI; EHEI-AgNPs were reported to have markedly improved biomedical characteristics than EHEI extract.

This remarkable qualities could lead to the identification of antioxidant and cytotoxic drugs from *Isochrysis* sp., and it propose that algal-based nanotechnology might optimize the therapeutic delivery of nanoparticles without eliciting adverse effects.

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