ORIGINAL ARTICLE

Characterization and Biological Activity of Silver Nanoparticles Synthesized from *Sargassum aquifolium*: A Sustainable Approach

[1]Reham Rabei*, [2]Mustafa M. El-Zayat, [1]Omar A. El-Shahaby, [1]Eladl Eltanahy

[1]Botany Department, Faculty of Science, Mansoura University, Mansoura 35516, Egypt.

[2]Unit of Genetic Engineering and Biotechnology, Faculty of Science, Mansoura University, Mansoura 35516, Egypt; Biology Department, New Mansoura University, New Mansoura, Dakahlia Governorate 35742, Egypt.

Key words:
Sargassum aquifolium, antioxidant, antimicrobial, fatty acid, green nanotechnology

Corresponding Author:
Reham Rabei
Botany Department, Faculty of Science, Mansoura University, Mansoura 35516, Egypt
Tel.: 01092902670
motawehreham@gmail.com

ABSTRACT

Background: *Algal extracts provide efficient route for green synthesis of nanoparticles.* ***Objectives****: This research aims to biosynthesize silver nanoparticles (AgNPs) using Sargassum aquifolium extract and to evaluate their antioxidant and antimicrobial potential.* ***Methodology****: The synthesis was initiated by the reduction of silver ions with functional groups in S. aquifolium extract. The antimicrobial and antioxidant activities were tested using well diffusion assay DPPH assay.* ***Results****: UV-Vis spectrometry revealed absorption maxima at 521 nm for the synthesized AgNPs, compared to 470 nm for the algal extract. Transmission electron microscopy indicated that AgNPs were spherical or pentagonal, with a size ranging from 6.48 to 9.80 nm and a zeta potential of -15.6 mV, indicating relative stability. Fourier transform infrared spectroscopy affirmed the involvement of certain groups in the capping and stabilization of the AgNPs. There was a decrease in total phenolics and flavonoids in the formed nanocomposite than the main algal extract due to their involvement in the reduction process. The algal extract and nanocomposite formed expressed significant antioxidant scavenging activity. The antimicrobial efficacy of the extract and AgNPs were evaluated against various microbial pathogens that are Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Bacillus subtilis, Escherichia coli, Klebsiella pneumonia, and Candida albicans. The results revealed broad antimicrobial spectrum against all the screened species. Additionally, fatty acid analysis identified palmitic acid, oleic acid and linoleic acid as the predominant fatty acids.* ***Conclusion****: These findings underscore the importance of S. aquifolium as a source of natural antimicrobials and in green nanotechnology applications.*

INTRODUCTION

Marine macroalgae (seaweeds) are increasingly recognized as valuable marine commodities due to their rich content of bioactive constituents and significant biological effects[1]. Recent research highlights the diversity of marine organisms as a source of bioactive constituents, that contribute to their importance[2,3]. Seaweeds are characterized by their morphological diversity, life cycles, and the production of various secondary metabolites, including antimicrobial and cytotoxic agents such as polysaccharides, tannins, sterols, alkaloids, cyclic peptides, glycerol-lipids, diterpenoids, and quinones. These constituents exhibited the capability of inhibiting the growth of many bacterial pathogens, leading to increased interest in the chemistry of marine algae for potential pharmaceutical applications[4].

Within the littoral zone of the Egyptian coast, brown algae presently stand as the preeminent group. The brown marine macroalgae, present in the Red Sea in Egypt, primarily consists of water (up to 90%) and polysaccharides, like cellulose, alginic acid, laminarin, and fucoidan. They also contain fatty acids, proteins, pigments, vitamins, sterols and terpenoids in addition to exhibiting the highest concentrations of phenolics among marine algae[5].

S. aquifolium found to be rich in phenols, steroids, alkaloids, and terpenoids that serve as antimicrobial agents, expressing anti-bacterial potential against biofilm-forming human pathogenic bacteria. *S. aquifolium* extract expressed activity against *Pseudomonas fluorescens*[6].

Greenly biosynthesized nanoparticles have attracted significant attention due to their safe biological activities, possessing promising characteristics and extensive applications across various domains. Additionally, their environmentally friendly characteristics increase their attractiveness[7]. Marine algae are considered important sustainable marine resources, and their capacity to produce nanoparticles has garnered significant interest because of their cost-effectiveness and ease of scalability in production[8-9].

The aim of this study was to biosynthesize silver Nano-composites using S*argassum aquifolium* and to

characterize the greenly synthesized silver nanoparticles and to compare the algal extract with the synthesized Nano-composites regarding their chemical composition, antioxidant activity, antimicrobial activity in addition to the identification of fatty acids content using gas chromatography-mass spectroscopy (GC-MS).

METHODOLOGY

The macro-algal species, *Sargassum aquifolium* as indicated in Fig (1), was collected in December 2020 from the Red Sea along the coast of Hurghada, Egypt (27°07'20.64"N; 34°50'41.46"E), during low tides (Fig 2). Macroalgae were harvested at maturity and kept in polyethylene bags containing seawater then washed with distilled water, to eliminate salts and residues, air dried and ground into a fine powder[10].

Fig.1: The seaweed sample collected (*Sargassum aquifolium*).

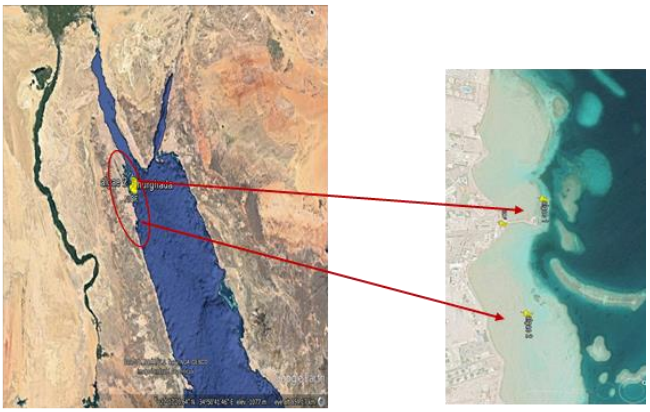


Fig 2.: Map depicting the sampling site along the Hurghada coastline.

Extraction:

10 grams of dried powder from algal species were soaked in 100 ml 30% methanol, followed by shaking at room temperature for 3 hours at 240 revolutions per minute, then filtered and the stock solution of the algal extract was kept for subsequent analysis following the methodology of **Algotiml *et al.*[11]**.

Synthesis of AgNPs utilizing studied marine algae extracts:

A modified version of the methodology of **El-Ghamry et al.[12]** proposed by **Dent *et al.*[13]** was employed to extract the active components from the chosen seaweeds, *S. aquifolium*. 100 grams of dried *S. aquifolium* were extracted using one liter of distilled water at 70 degrees Celsius for 2 hours. An equivalent volume of the algal extract was stirred while 1 L of a 1 mmol aqueous solution of $AgNO_3$ was added dropwise and stirred for 2 hours[14-15]**.** The nanoparticles were subsequently transferred to the ultraviolet (UV) irradiation apparatus and subjected to UV radiation for 20 minutes. The synthesized nanoparticles were kept at 4°C[16].

Characterization of AgNPs:

The synthesized AgNPs seaweed extract was characterized using UV-visible spectroscopy, Fourier transform infrared (FTIR), transmission electron microscopy (TEM) and zeta potential analysis.

Bioactive constituents:

Total phenols:

According to Limmongkon *et al.*[17], Folin–Ciocalteau method was used to estimate total phenolics. 0.5 ml sample, 0.1 ml Folin reagent, and 0.5 ml 7.5% Na_2CO_3 solution were mixed and completed to 10 ml volume. The absorbance was detected at 740 nm using gallic acid as a standard.

Total flavonoids:

Aluminum chloride method modified by Munhoz et al, [18] was used to measure flavonoids. 0.5 ml sample was combined with two ml MeOH, 0.2 ml CH_3COOK (1M), 0.3 ml 10% $AlCl_3.6H_2O$ solution, and two ml distilled water, then absorbance was measured at 430 nm using quercetin dihydrate as a standard.

Antioxidant Activity using DPPH radical scavenging assay:

DPPH solution (60 micromolars) was added to the samples at a ratio of three parts DPPH to one part sample and the absorbance was measured at 517 nm after 30-minute incubation in dark. Ascorbic acid was used as standard for comparison[19].

DPPH scavenging activity was calculated as follow:

$$\text{DPPH scavenging activity} = \frac{\text{A Con.} - \text{A S.}}{\text{A Con.}} x\ 100$$

Where: $A_{con.}$ = absorbance of DPPH solution, A_s = absorbance of DPPH and samples.

Antimicrobial test utilizing the Agar Disc Diffusion Assay:

The antibacterial potential was screened using *Staphylococcus aureus* and *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella* and *Escherichia coli* in addition to the fungal strain *Candida albicans*.

The disk diffusion assay was conducted for screening of the antimicrobial activity[20]. A 6-mm diameter filter paper discs were soaked in the samples for 2-3 hours then dropped on the agar plates seeded with the tested strains and incubated at 37°C for 37 hours. Chloramphenicol at a concentration of 500 mg/ml used as positive control, while the respective solvents acted as negative controls. The described procedures are applicable for fungal assays, utilizing Sabouraud Dextrose Agar (SDA) media[21]. Penicillin (500 mg/ml) served as the standard, while the solvents of each extract acted as negative controls. The diameter of inhibition zones formed by the samples, were measured.

GC-MS analysis

The algal extract was analyzed by gas GC-MS (Thermo-Scientific TRACE-1310 GC coupled with ISQLT single quadrupole mass spectrometer, electron impact, 70 eV) at the National Research Center, Egypt. A DB5-MS column was employed with a helium flow rate of 1 mL/min as the carrier gas. The WILEY and NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA) libraries were utilized to identify the principal peaks[22].

RESULTS

Characterization of nanoparticles:

AgNPs biosynthesis and verification using UV-VIS spectroscopy:

The creation of AgNPs commences upon reduction of silver ions with *S. aquifolium* extract. The reduction was apparent from the color change to brownish-yellow almost after 48 hours (Fig.3). The UV–Vis spectra of AgNPs generated from the extract presented in (Fig.4). The absorption maxima of AgNPs, that stabilized by *S. aquifolium*, was observed at 521 nm. While the absorption maxima of normal *S. aquifolium*, was observed at 470 nm.

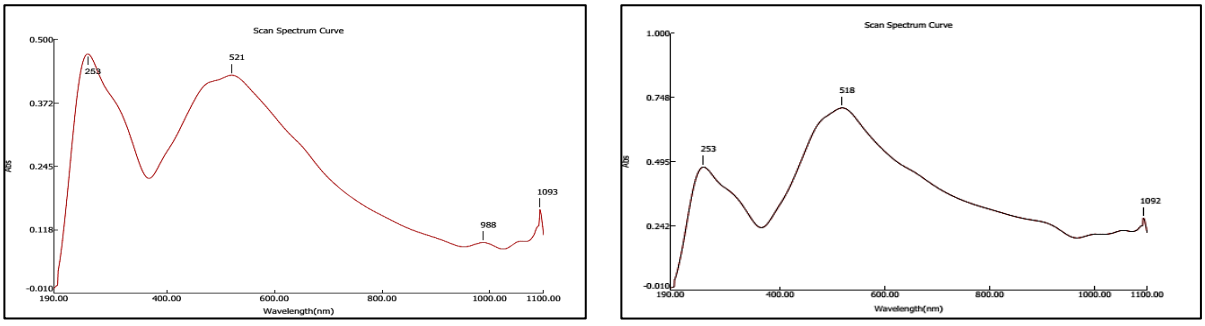
Fig. 3: AgNPs synthesis at 0 h and after AgNPs synthesis

TEM analysis:

Figure 5 presents TEM images of AgNPs, that were spherical or pentagonal, well distributed, and with size between 6.48 to 9.80 nm.

Zeta potential:

Silver nanoparticles capped by *S. aquifolium* expressed relative stability as they exhibited zeta potential values of -15.6 mV (Fig. 6).



(A) AgNPs capped by *S. aquifolium* (B) *S. aquifolium* extract

Fig. 4: UV–vis spectra of the synthesized AgNPs (a) and *S. aquifolium* extract (b)

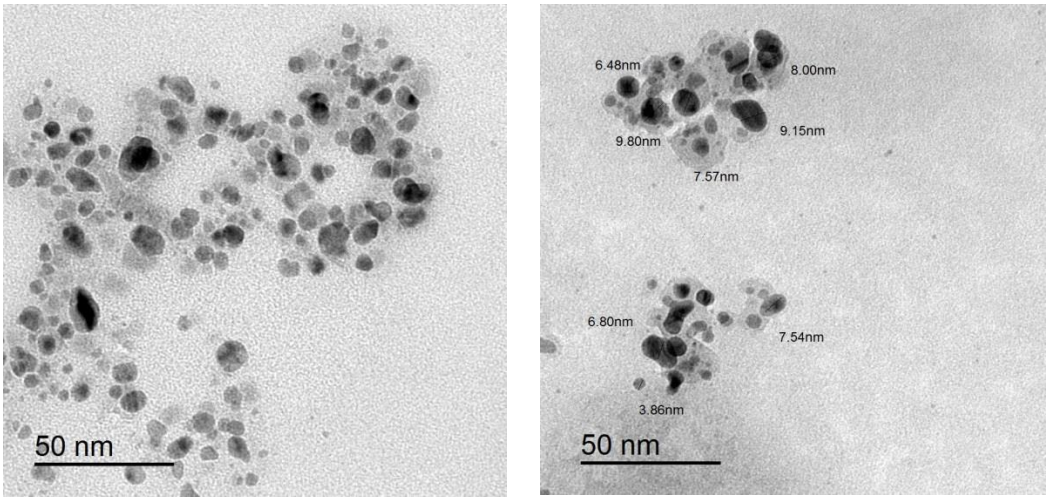


Fig. 5: TEM images of the synthesized AgNPs using *S. aquifolium*

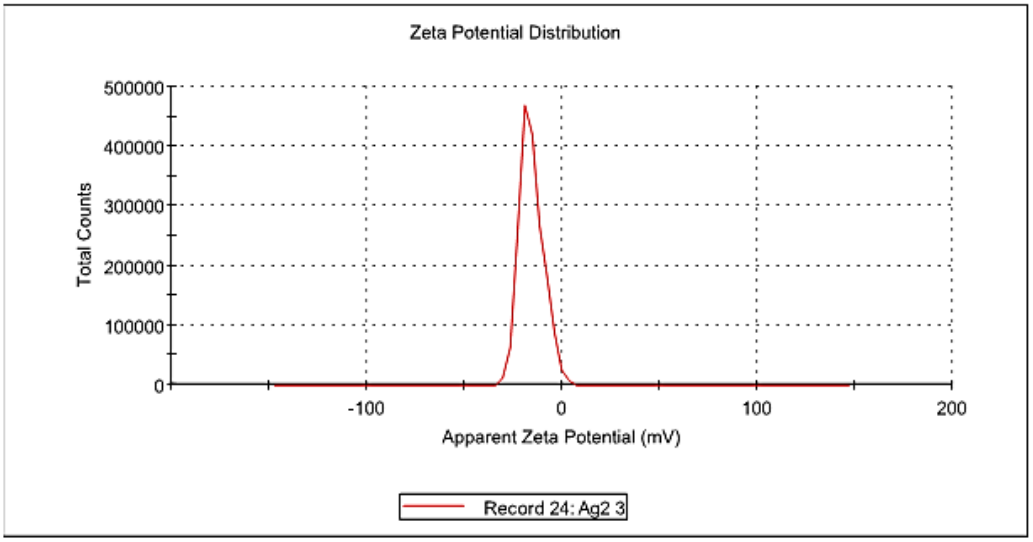
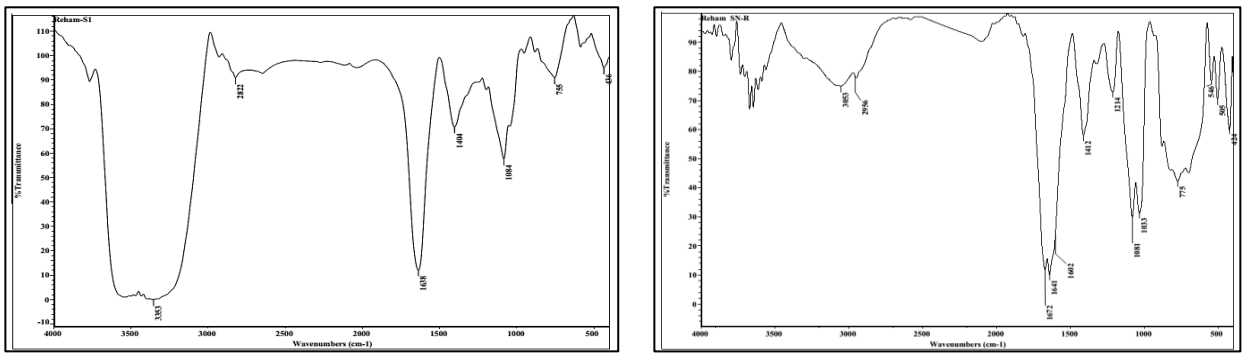


Fig. 6: Zeta potential for the AgNPs capped by *S. aquifolium*

FTIR analysis:

The FTIR spectra of *S. aquifolium* extract and the biosynthesized AgNPs are presented in (Fig. 7). The peaks of algal extract suggest the participation of its functional groups in nanometal synthesis. Additionally, the presence of certain peaks in the spectra of nanoparticles illustrates their contribution in capping and stabilizing the synthesized AgNPs. The wavenumber range in which FTIR spectra were obtained was 4000–500 cm^{-1}. Smaller peaks within the range of 550 cm^{-1} to 1700 cm^{-1} correspond to the functional groups, C-N, COOH, C-C, C-O, and C-O-C moieties. The *S. aquifolium* extract (Fig.7a) exhibited a peak at 3353 cm^{-1} for hydroxyl group O–H stretching vibrations. The peak at 1638 cm^{-1} revealed C=O stretching vibration and1404 cm^{-1} for C-O- vibration. Figure7b shows the spectrum of AgNPs capped by *S. aquifolium*. The absorbance band observed at approximately 3053 cm^{-1} represent C-H stretching, 2956 cm^{-1} for C-H stretching vibrations. The three peaks at 1602, 1641 and 1672 cm^{-1} depicted the C-O vibrations. The absorption band at 1214 cm^{-1} represents N-C vibration.



(A) *S. aquifolium* normal **(B) AgNPs capped by *S. aquifolium***

Fig. 7: FTIR spectra of *S. aquifolium* extract (a) and AgNPs capped by *S. aquifolium* (b)

Phytochemical analysis:

Bio active compound:

The average values for phenolics and flavonoids of *S. aquifolium* extract were 13.33 mg gallic acid equivalent (GAE)/g dry extract and 7.86 mg quercetin equivalent(QE)/g, while in nano- *S. aquifolium* composite 9.43 mg (GAE)/g and 5.16 mg QE/g, respectively.

Antioxidant scavenging activity:

The DPPH assay is a conventional technique employed to assess the overall antioxidant capability in natural extracts. The results are expressed as IC50 values, indicating the quantity of antioxidants required to diminish the initial DPPH concentration by 50%. The antioxidant activities of crude and silver nano composites were assessed by measuring DPPH radical scavenging activity at different concentrations, as detailed in Table 1. The DPPH radical scavenging activities of all tested extracts exhibited a dose-dependent relationship, increasing with higher extract concentrations (Fig.8).

S. aquifolium exhibited an IC50 of: 244.85 µg/ml, while that of the nano-silver *S. aquifolium* composite was: 281.61 µg/ml. These results revealed good antioxidant activity in comparison with ascorbic acid that has IC50 of 23.3 µg/ml and substantial difference between the extract and nano composite.

Table 1: Radical scavenging activity (%) of the tested samples

Concentration µg/ml	DPPH radical scavenging	
	Extract	Nanocomposite
25	7.11	4.42
50	10.21	7.38
100	14.08	10.29
IC_{50}	244.85	281.61

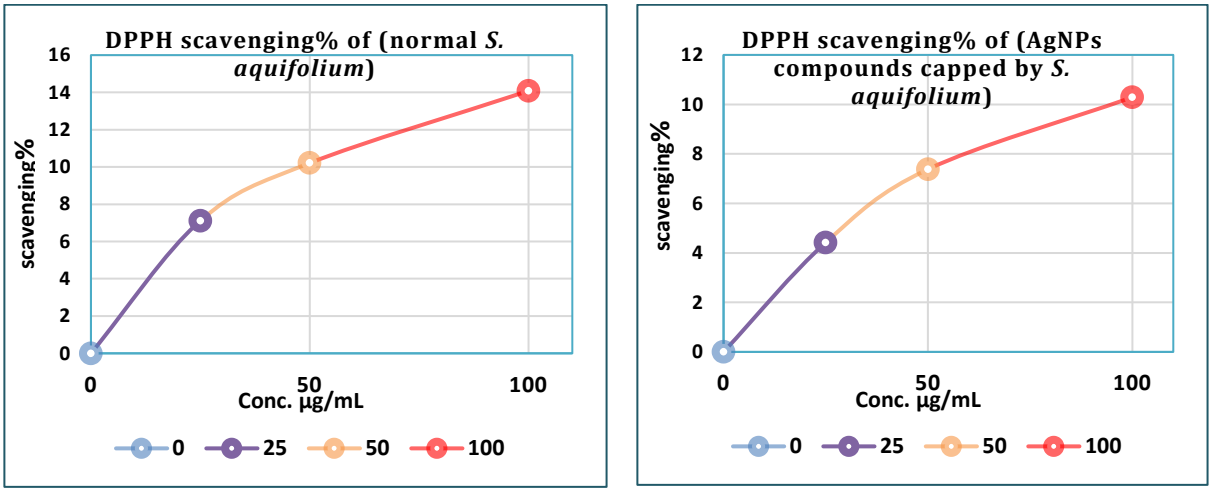


Fig. 8: DPPH radical scavenging activity (%) of investigated *S. aquifolium* extracts

Antimicrobial activity:

The disc diffusion assay was used to elucidate the antimicrobial potential of *S. aquifolium* and the synthesized silver nanocomposite. The antibacterial activity was screened using *S. aureus, B. subtilis, P. aeruginosa, K. pneumonia, S. typhi* and *E. coli* bacterial strains. The antifungal activity was screened using *C. albicans*. Distinct inhibition zones were detected as illustrated in table 2. The synthesized nanoparticles expressed potent antimicrobial activity in comparison with the standard antibiotics used.

GC-Mass analysis:

Fatty acids are among the primary constituents of human diet. All the identified components are presented in Table (3) and Fig. 9 that demonstrate the fatty acids present in *S. aquifolium.* The analysis revealed that palmitic acid (34.21%), oleic acid (18.18%) and linoleic acid (17.06%) were the predominant among the identified fatty acids.

Table 2: Antimicrobial activity of *S. aquifolium* extracts and silver nano-composite

Pathogenic strains	*S. aquifolium*	Silver Nanocomposite	Chloramphenicol	Penicillin
S. typhi	13	14.5	13	12
P. aeruginosa	14	16	14	13
C. albicans	9	10	11	11
B. subtilis	11	17	15	15
S. aureus	14	16	15	13
K. pneumonia	10	15	14	14
E. coli	11	18	14	14

Table 3: Fatty-acid composition of *S. aquifolium*.

Fatty acids	Percent
Myristic acid	3.53%
Pentadecanoic acid	0.81%
Palmitic acid	34.21%
Stearic acid	2.30%
Oleic acid	18.18%
Vaccinic acid	1.29%
Linoleic acid	17.06%
Linolenic acid	4.39%
Stearidonic acid	1.03%
Arachidonic acid	9.79%
Eicosapentaenoic acid (EPA)	2.10%

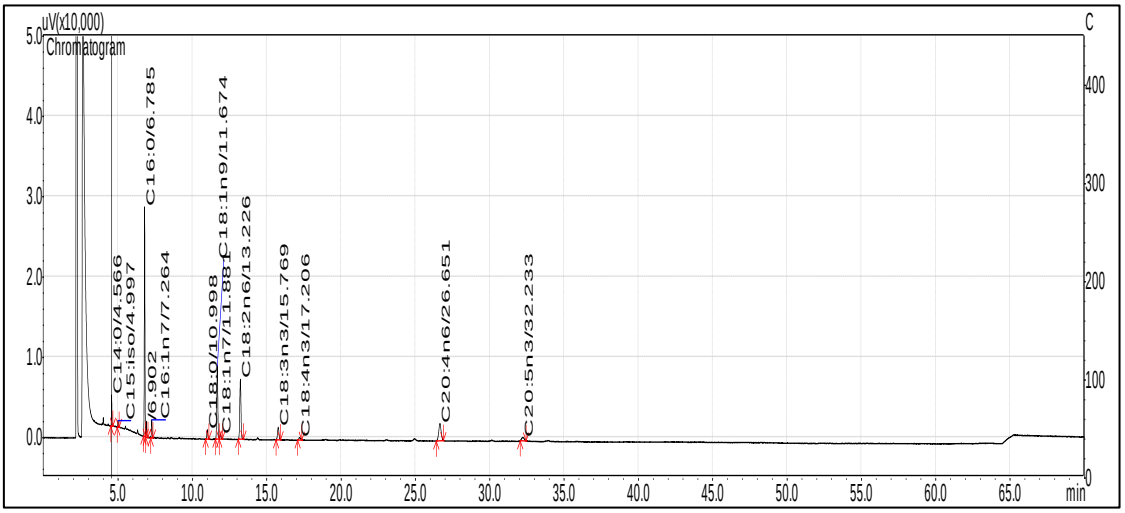


Fig. 9: The GC-MS chromatogram of the investigated *S. aquifolium*

DISCUSSION

Seaweeds, or macroalgae, are recognized for their rich diversity and significant contributions to ecological health and human welfare. They produce a variety of bioactive compounds that exhibit numerous biological activities, making them valuable in pharmaceuticals, food, and agriculture. This research discussed the biosynthesis of AgNPs using *S. aquifolium*, their phytochemical properties, and the applications of these compounds as antioxidants and antimicrobials[23,24].

The greenly synthesized AgNPs using *S. aquifolium* extract is a sustainable approach that utilizes the natural compounds found in seaweeds extracts, that can reduce AgNO3 to form AgNPs, which are characterized by a characteristic brownish-yellow color due to surface plasmon resonance. The synthesis process involves electrostatic interactions between silver ions and compounds containing reducing groups like hydroxyl and amino groups, in *S. aquifolium* extract, that act as reducing and stabilizing agents[24,25].

Characterization of AgNPs was done using UV-Vis spectroscopy, FTIR analysis, TEM, and zeta potential analysis and the obtained results confirmed the successful synthesis and stability of them. The zeta potential values indicate that the AgNPs exhibit moderate stability, which is essential for their applications in different fields[26,27].

The furious phenolics, flavonoids, saponins, and polysaccharides content in seaweeds extracts are linked to many biological applications like antimicrobial and antioxidant activities[28]. These compounds impact reduction process contributes to the synthesis of silver nanoparticles[29].

The antioxidant capacity of *S. aquifolium* and the prepared silver-nanocomposite was evaluated using the DPPH assay. Previous studies indicated that brown seaweeds generally possess higher antioxidant potential compared to other algal varieties, correlating with their phenolic content[30].

The antimicrobial efficacy was screened against various pathogens, including pathogenic bacterial and fungal strains. The results demonstrate broad antimicrobial spectrum against all the screened pathogens including *S. aureus, B. subtilis, P. aeruginosa, K. pneumonia, S. typhi, E. coli* and *C. albicans.* This highlights the potential of AgNPs as safe and effective alternatives to traditional antibiotics, suggesting their use as natural treatments for antibiotic-resistant bacterial infections[31,32].

The mechanism of action for AgNPs involves disrupting bacterial membranes, generating reactive oxygen species, and inhibiting essential cellular functions, which contributes to their effectiveness against resistant strains[33,34].

GC-MS analysis of seaweed extracts reveals a diverse array of bioactive compounds, particularly fatty acids, which are essential for various biological functions. The polyunsaturated fatty acids present in seaweeds are known to possess many health benefits, including cardiovascular protection and immune system enhancement[35,36]. The lipid content and fatty acid profile are affected by multiple factors, including species, environmental conditions, and extraction methods[37]. The obtained results indicated 13 fatty acids present in S. *aquifolium dominated by palmitic acid followed by* Oleic acid and Linoleic acid while the lowest content was for Pentadecanoic acid.

CONCLUSION

In conclusion, seaweeds are a valuable source of phytochemical compounds that contribute in many important biological applications. The synthesis of silver nanoparticles (AgNPs) using *Sargassum aquifolium* extract demonstrates the potential of this seaweed in the field of green nanotechnology. Ongoing research into the phytochemical composition and biological activities of *S. aquifolium* will further expand its applications in medicine, food preservation, and sustainable practices. Additionally, further studies on the use of greenly synthesized nanomaterials are essential to establish them as natural alternatives to antibiotics for treatment purposes.

Declarations:
Consent for publication: Not applicable
Availability of data and material: Data are available upon request.
Competing interests: The author(s) declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.
This manuscript has not been previously published and is not under consideration in another journal.
Funding: Authors did not receive any grants from funding agencies.

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