ORIGINAL ARTICLE

# Green synthesis of Silver Nanoparticles using *Cystoseira trinodis*: A Sustainable Approach to develop and characterize natural Antimicrobial Drug

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## ABSTRACT

***Key words:***
***Cystoseira trinodis, Silver nanoparticle, antioxidant, fatty acids, pathogenic microbial strains, Antimicrobial drugs***

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***Background**: Cystoseira trinodis extract provide efficient route for green synthesis of silver nanoparticles due to its furious phytochemical content. **Objectives**: This research aims to biosynthesize silver nanoparticles (AgNPs) utilizing C. trinodis extract and to assess their biological activity. **Methodology**: The reduction of silver ions by the algal extract commence the biosynthesis of AgNPs and these nanocomposites were screened for their antioxidant and antimicrobial potential. **Results**: The formation of AgNPs confirmed by maximum absorption peak at 524 nm using UV-Vis spectroscopy, spherical black spots of AgNPs presented using transmission electron microscope, with an average size of 6.12 to 14.92 nm and a zeta potential of -17.2 mV that suggests that AgNPs are relatively stable. The analysis conducted using Fourier-transform infrared spectroscopy validated the participation of functional groups in the capping and stabilization of the nanoparticles. The algal extract exhibited higher total phenolic and flavonoid contents than that of nanocomposite solution due to usage of these groups in the synthesis of nanoparticles. The extract and the formed nanocomposites were screened against the pathogenic microbial strains, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumonia, Salmonella typhi, Escherichia coli, and Candida albicans and the synthesized AgNPs expressed strong antimicrobial efficacy in comparison with conventional antibiotics. Furthermore, the phytochemical screening revealed that palmitic acid was the most prevalent fatty acid (28.02%), followed by oleic acid (26.77%) and linoleic acid (22.29%). **Conclusion**: The results highlight, C. trinoids is promising in a green synthesis of silver nanoparticles that could be used as natural antimicrobial drug.*

## INTRODUCTION

One of the most significant global threats is antimicrobial resistance[1,2]. An increasing number of antibiotic-resistant organisms and novel resistance mechanisms are evolving and disseminating worldwide, jeopardizing our capacity to treat prevalent infectious diseases. Antibiotics are becoming less effective, making it more difficult to treat a growing number of infections[3]. Antibiotics are still essential for treating some bacterially based infectious disorders, though. Recently, there has been a significant interest in the reliance on secondary metabolites derived from natural sources for the management of various ailments and the defense against numerous conditions[4-6]. Finding substitutes that can lessen the usage of antibiotic medications is therefore essential.

Macroalgae, commonly known as seaweeds, represent a diverse collection of multicellular marine organisms that demonstrate remarkable adaptability to the challenging conditions of marine environments through the production of distinctive natural compounds[7]. In particular, they are recognized for their bioactive substances, including proteins, lipids, polysaccharides, and polyphenols, which are responsible for the antibacterial, antiviral, and antifungal properties of these organisms[8]. Seaweeds are distinguished by their diverse morphologies, life cycles, and generation of a range of secondary metabolites, including cytotoxic and antibacterial substances such cyclic peptides, polysaccharides, sterols, tannins, alkaloids, glycerol-lipids, diterpenoids, and quinones. The compounds in question have demonstrated the ability to inhibit the growth of a range of Gram-positive and Gram-negative bacterial pathogens, resulting in heightened interest in the chemistry of marine algae for potential pharmaceutical applications[9].

Brown algae are currently the most prevalent group in the littoral zone of the Egyptian coast. An extensive amount of research has been conducted on the brown algae of the *Cystoseira* family, which are derived from marine macroalgae that are present in the Red Sea in Egypt[10]. Terpenoids, polysaccharides, alkaloids, and steroids are among the many compounds that have been identified from different species of the Mediterranean brown algae of the genus *Cystoseira*; however, there aren't many published studies on the pharmacological properties of these compounds and other *Cystoseira* species[6,11-12].

*Cystoseira trinodis* exhibited the highest concentrations of unsaturated fatty acids, primarily consisting of oleic acid. Unsaponifiable lipids are predominantly composed of phytol, cholest-5-en-3-ol, stigmasterol, and 9Z-octadecenamide. Gallic acid was the main phenolic component, with a value of 167.18 µg/g. The main non-essential AA was proline, while the main essential AAs were leucine and phenylalanine[13]. The methanol extracts of *C. trinodis* exhibited a modest antioxidant activity, characterized by IC50 values of 30.5 µg/ml. The FAs found in *C. trinodis* had antibacterial properties against *Bacillus cereus* and *Staphylococcus aureus*. In addition, *C. trinodis* exhibited potent antibacterial activity against *Salmonella typhimurium*, *B. subtilis*, *E. coli* and *Aspergillus fumigatus*[14].

Recently, nanotechnology has developed into a unique technological field, presenting various applications in medicine, agriculture, and industry[15]. Because of their safe biological activity, green biosynthesized nanoparticles have garnered a lot of interest, possessing promising characteristics and extensive applications across various domains. Additionally, their environmentally friendly characteristics increase their attractiveness[16]. Marine algae are recognized as vital sustainable marine resources, and their ability to generate nanoparticles has attracted considerable attention. Algal biosynthesized nanoparticles are preferred over traditional methods due to their cost-effectiveness and scalability in production[17]. At low doses, AgNPs from *Cystoseira baccata* and *Cystoseira tamariscifolia* showed the highest bactericidal and bacteriostatic effects against *E. coli* and *P. aeruginosa*[18].

The current study aims to biosynthesize silver nanoparticles using *Cystoseira trinodis*, characterize the greenly synthesized silver nanoparticles, compare the chemical composition, antioxidant activity, and antimicrobial activity of the algal extract and the synthesized nanocomposites, and use gas chromatography-mass spectroscopy (GC-MS) to identify the bioactive compounds in the marine algae.

## METHODOLOGY

**Sample collection**

The macroalgal species *Cystoseira trinodis* was collected in December 2020 from the Red Sea near Hurghada, Egypt at maturity, in polyethylene bags then cleaned with tap water, air-dried, and crushed into a fine powder[19].

**Extraction:**

The methanolic extract was obtained by immersing 10 g of dried algal powder in 100 ml of 30% methanol, followed by shaking at room temperature for 3 hours at 240 rpm. Following this, the mixture underwent filtration utilizing Whatman paper No. 1. A stock solution of the algal extract was prepared for subsequent analysis[20].

**Utilization of marine algae extracts for the synthesis of silver nanoparticles:**

One liter of distilled magnetized water was employed to extract 100 grammes of algal powder at 70 degrees Celsius for two hours. Additionally, AgNO3 sourced from El-Gomhoria Company was employed to synthesize nanoparticles using an environmentally friendly method[21-23]. An equivalent volume of the algal extract was stirred while 1 L of a 1 mmol aqueous solution of $AgNO_3$ was added gradually. The mixture was stirred for an additional two hours at room temperature after the complete incorporation of the metal aqueous solution[24]. The nanoparticles were then placed in the ultraviolet (UV) irradiation apparatus and exposed to UV radiation for 20 minutes[25].

**Nanoparticles characterization:**

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

**Bioactive constituents:**

**Total phenols:**

Folin-Ciocalteau assay was performed to determine the total phenolics. The reaction mixture consists of 0.5 mL methanolic extract, 0.1 mL Folin reagent, and 0.5 mL 7.5% $Na_2CO_3$ solution. The absorbance at 740 nm was measured after an hour of incubation at room temperature in the dark. The experiment was carried out in triplicate. Gallic acid was employed as a standard [26].

**Total flavonoids:**

The aluminum chloride colorimetric method was employed with minor adjustments to quantify the total flavonoid concentration. 0.5 ml of MeOH extract was mixed with 2 ml of MeOH, 0.2 ml of 1M CH3COOK, 0.3 ml of 10% AlCl3.6H2O solution, and 2 ml of purified water. Absorbance was recorded at 430 nm after a 30-minute incubation at ambient temperature. Quercetin dihydrate served as the reference for flavonoid content[27].

**Antioxidant Activity using DPPH radical scavenging assay:**

Sixty micromolar of DPPH methanolic solution was added to the prepared samples at a ratio of three parts DPPH to one part extract. Following a 30-minute incubation at exclusion of light, the absorbance was assessed at 517 nm. The antioxidant activity of each sample was compared to that of the standard ascorbic acid[28].

Where control is the absorbance of DPPH and MeOH instead of the sample, Sample is the absorbance of DPPH and investigated samples.

**Antimicrobial test utilizing the Agar Disc Diffusion Method:**

The antimicrobial activity was screened using the bacterial strains *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Salmonella* and *Escheria coli* in addition to the fungal strain *Candida albicans*. The disc diffusion assay was performed to evaluate antibacterial activity[29,30].

**GC-MS analysis**

The analysis of the algae extracts was conducted through gas chromatography–mass spectrometry, utilizing the Thermo Scientific TRACE 1310 GC in conjunction with the ISQLT single quadrupole mass spectrometer, employing electron impact at 70 eV, at the National Research Centre in Egypt[31].

## RESULTS

**Characterization of nanoparticles:**

**AgNPs biosynthesis and verification using UV-VIS spectroscopy:**

The creation of AgNPs commences when the silver source engages with the functional groups present in the extract. This process induces the reduction of silver, leading to the formation of AgNPs. The reduction of $AgNO_3$ by *C. trinodis* was clearly apparent from the color alteration of the reaction mixture (brownish-yellow) after 48 hours (Fig 1). The brown hue's intensity was directly associated with the incubation period. The AgNPs' UV-Vis spectra produced by extracting two different types of algae are presented in Fig. 2. The absorption maxima of AgNPs stabilized by *C. trinodis* were showed at 524 nm. While the absorption maxima of normal *C. trinodis* were showed at 518 nm.

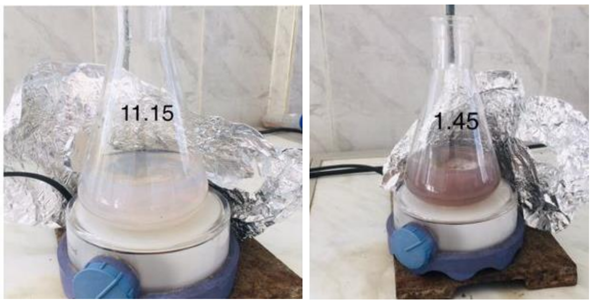


**Fig. 1:** The production of silver nanoparticles at 0 and 48 hours prior to and following heating.

**TEM analysis:**

Fig. 3 displays TEM images of AgNPs stabilized by *C. trinodis*. TEM pictures of AgNPs stabilized by *C. trinodis* exhibit spherical shapes, grey colouration, and black spots, exhibiting an average particle size between 6.12 and 14.92 nm.

**Zeta potential:**

The zeta potential method evaluates zeta potentials, which vary from þ100 to -100 mV. Silver nanoparticles capped by two marine algal extracts, specifically *C. trinodi*, demonstrated relative stability, exhibiting zeta potential values of -17.2 mV (Fig. 4).

**(A) AgNPs capped by *C. trinodis***　　**(B) *C. trinodis* extract**

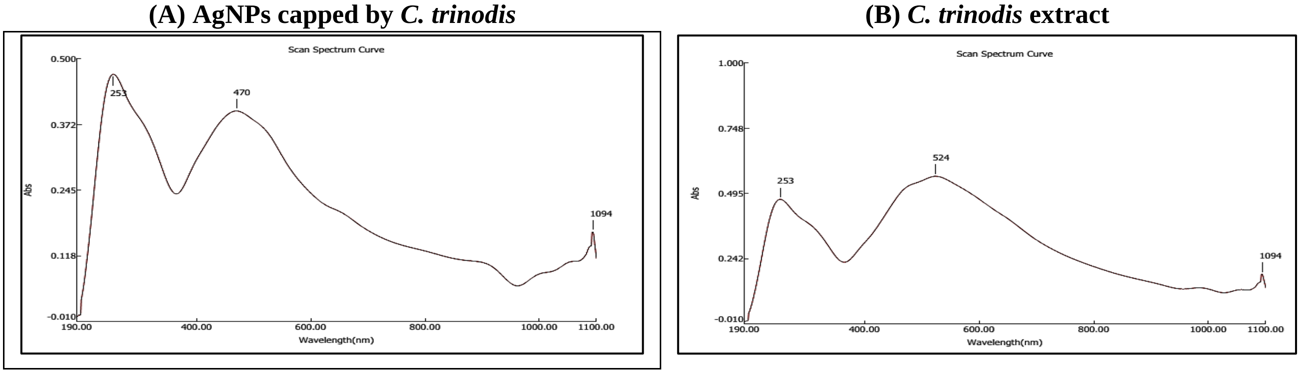


**Fig. 2:** UV–vis spectra of *C. trinodis*-capped biosynthesised AgNPs' (a) and control (b

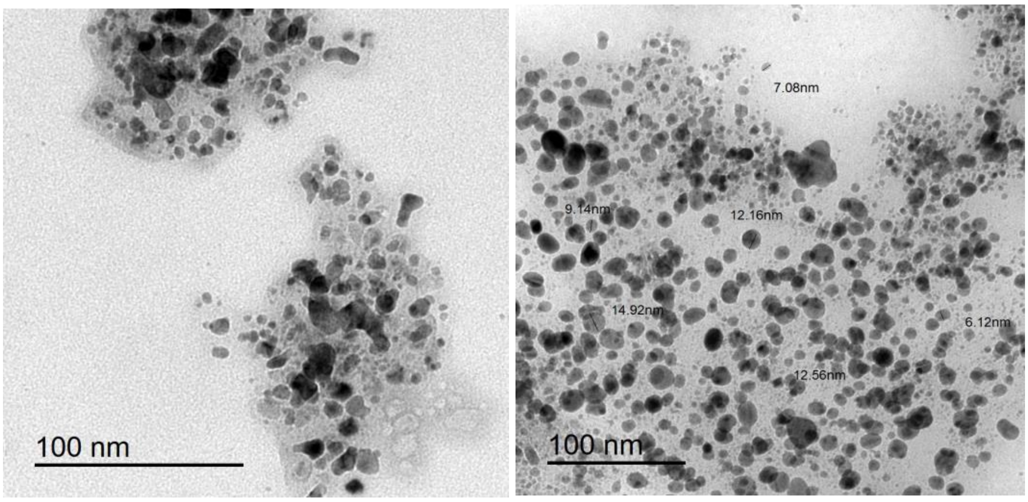


**Fig. 3:** TEM pictures of biosynthesized AgNPs stabilized by marine algal extract of *C. trinodis*

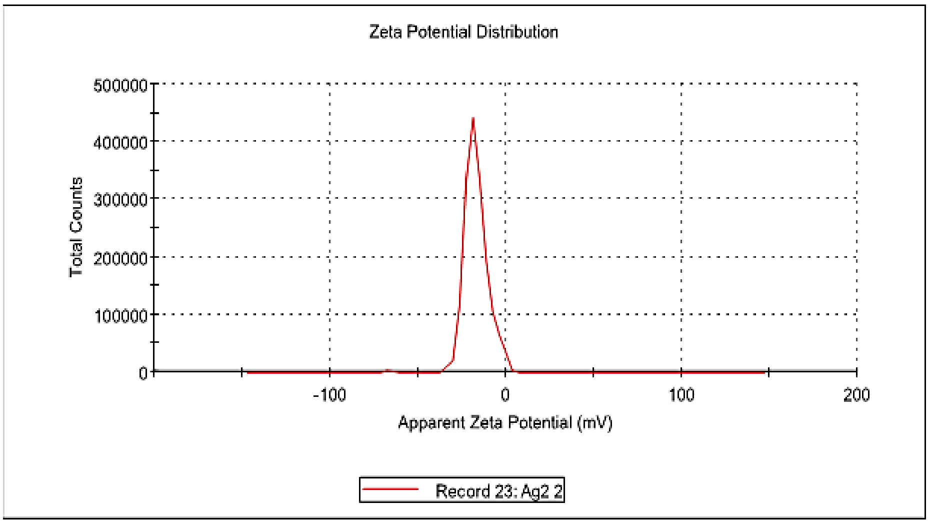


**Fig. 4:** Zeta potential for the AgNPs capped by *C. trinodis*

**FTIR analysis:**

The usual *C. trinodis* infrared spectra (Fig. 5) displayed an O–H stretching vibration band at 3492 $cm^{-1}$. The two bands observed at roughly 2940 $cm^{-1}$ and 2797 $cm^{-1}$ were determined to be the asymmetric and symmetric stretching vibrations of CH2. The stretching vibrations associated with the C=O group were characterized by the band observed at 1639 $cm^{-1}$. The C-O-stretching vibration was identified as the peak observed at 1408 $cm^{-1}$, while the C-F group vibrations were identified as the peak at 1082 $cm^{-1}$. The stretching vibration of CH2 was described by the absorption band at 1038 $cm^{-1}$, while the C-H vibration was thought to be represented by the band at 930 $cm^{-1}$. The peak at 884 $cm^{-1}$ was ascribed to the C-O-O-group, whereas the peak at 600 $cm^{-1}$ was ascribed to the vibrations of C-l stretching. The band at roughly 448 $cm^{-1}$ reflected the S-S group vibrations.

In comparison, O-H stretching is represented by peaks at 3608 $cm^{-1}$ in AgNPs capped by *C. trinodis*. The C-H stretching is responsible for the peaks at 2752 $cm^{-1}$. It was determined that the band at 2058 $cm^{-1}$ depicted the isothiocyanate vibrations of -NCS. The C-O-vibration was observed at 1410 cm−1, but the C=O stretching vibration was found to be the peak at 1611 $cm^{-1}$. The N-C was the cause of the band at 1222 $cm^{-1}$. The C-F stretch was the cause of the peak at 1082 $cm^{-1}$, whereas the peak at 886 $cm^{-1}$ corresponded to C-O-O-stretch. The band at approximately 746 $cm^{-1}$ presented the C-Cl stretching vibrations. The two peaks seen at 673 and 636 $cm^{-1}$ were interpreted as the CH bend vibration, lastly the two peaks at 592 and 499 $cm^{-1}$ corresponded to the C-l stretching group vibrations.

**(A) *S. aquifolium* normal**

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

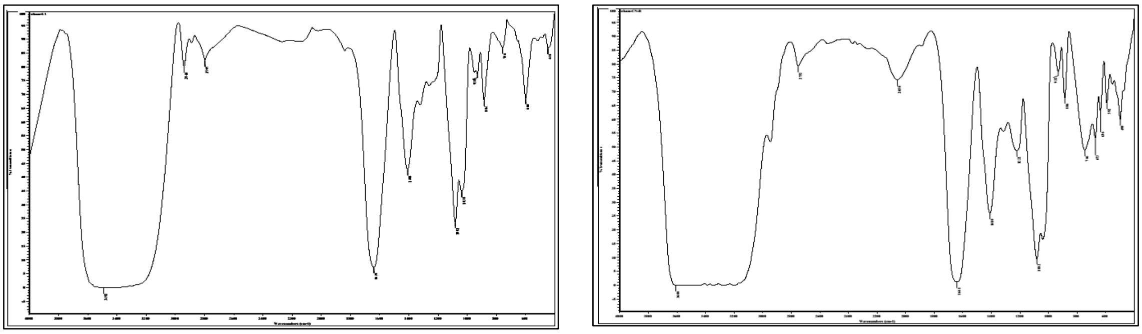

**Fig. 5:** FTIR spectra of *C. trinodis* extract (a) and FTIR spectra of AgNPs capped by *C. trinodis*

**Phytochemical analysis:**

**Bio active compound:**

The average values for total phenolic and flavonoid contents of the normal and nano seaweed extracts in the present study are presented in Table 1.

**Table 1: Bioactive compound of the investigated seaweeds.**

| Bio active compound / Extracts | Total phenolics (mg GAE/g) | Total flavonoids (mg QE/g) |
|---|---|---|
| **Normal *C. trinoids*** | 15.01 | 9.21 |
| **AgNPs-*C. trinoids*** | 11.07 | 6.58 |

**Antioxidant activity as DPPH radical scavenging activity:**

The DPPH radical scavenging 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 reduce the initial DPPH concentration by 50%. The antioxidant activities of crude and nano seaweed extracts were assessed by measuring DPPH radical scavenging activity at various concentrations (25, 50, and 100 µg/ml), as detailed in Table 2. The IC50 values for each seaweed extract were calculated and are also presented in the same table. The DPPH radical scavenging activities of all tested extracts exhibited a dose-dependent relationship, increasing with higher extract concentrations. The extracts from various seaweeds demonstrated differing antioxidant activities, as evidenced by the percentage of scavenging activity measured at the highest concentration of each crude and nano extract (Fig. 6).

The crude extract of the brown seaweed, *C. trinoids*, demonstrated an IC50 of 218.04 µg/ml, whereas the nano-silver *C. trinoids* composite exhibited an IC50 of 263.24 µg/ml. The results demonstrated significant antioxidant activity relative to ascorbic acid, which has an IC50 of 23.3 µg/ml, highlighting a notable difference between the extract and the nanocomposite.

**Table 2: DPPH radical scavenging activity (%) of investigated seaweeds extracts**

| Concentration µg/ml | *Inhibition of C. trinoids* | |
|---|---|---|
| | **Extract** | **Nano** |
| 25 | 8.51 | 5.17 |
| 50 | 11.68 | 9.08 |
| 100 | 16.24 | 12.34 |
| $IC_{50}$ | 218.04 | 263.24 |

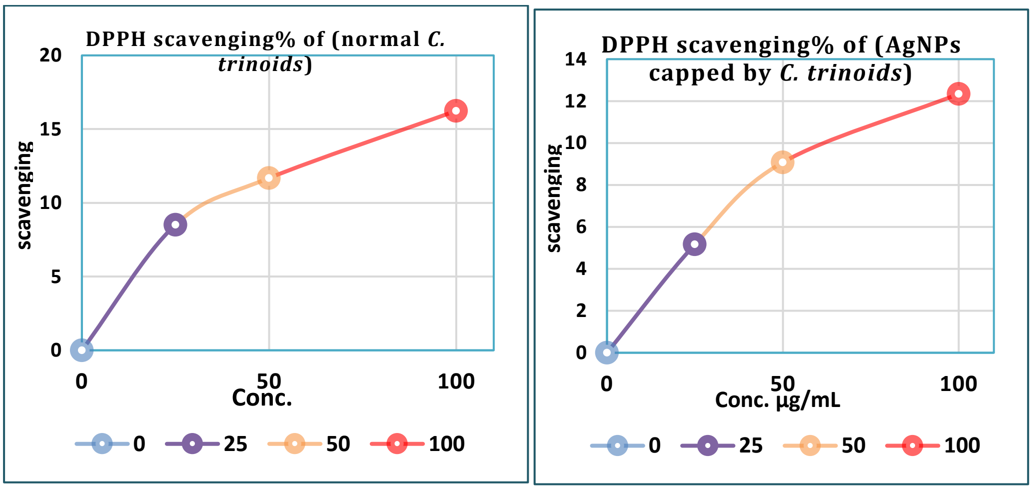


**Fig. 6:** DPPH radical scavenging activity (%) of ***C. trinoids*** extracts

**Antimicrobial activity:**

The synthesized nanoparticles demonstrated broad spectrum antimicrobial activity against all the tested pathogenic microbial strains when compared to the standard antibiotics employed. Distinct inhibition zones were observed, as demonstrated in Table 3. The results prove the effectiveness of the synthesized nanoparticles to be used as antimicrobial drugs.

**Table 3: Antimicrobial activity of *C. trinoids* extracts and silver nanocomposite**

| Pathogenic strains | *C. trinoids* | Silver Nanocomp | Chlora mphenic | Peni cilli |
|---|---|---|---|---|
| *S. typhi* | 12 | 15 | 13 | 12 |
| *P. aeruginosa* | 15 | 15.5 | 14 | 13 |
| *C. albicans* | 8 | 9 | 11 | 11 |
| *B. subtilis* | 15.5 | 16 | 15 | 15 |
| *S. aureus* | 14 | 17 | 15 | 13 |
| *K. pneumonia* | 9 | 14 | 14 | 14 |
| *E. coli* | 12 | 16 | 14 | 14 |

**GC-Mass analysis:**

Fatty acids represent essential components of the human diet. The identified components are detailed in Table 4 and illustrated in Fig 7, showcasing the fatty acids found in *C. trinoids*. Palmitic acid constituted the predominant fatty acid at 28.02%, followed by oleic acid at 26.77% and linoleic acid at 22.29%.

**Table 4: Fatty-acid composition of *C. trinoids*.**

| Fatty acids | Name | C. trinoids |
|---|---|---|
| C14:0 | Myristic acid | 2.37% |
| C15:0 | Pentadecanoic acid | - |
| C16:0 | Palmitic acid | 28.02% |
| C18:0 | Stearic acid | 4.05% |
| C18:1 ω9 | Oleic acid | 26.77% |
| C18:1 ω7 | Vaccinic acid | 2.03% |
| C18:2ω6 | Linoleic acid | 22.29% |
| C18:3ω3 | Linolenic acid | 3.93% |
| C18:4ω3 | Stearidonic acid | - |
| C20:4 ω6 | Arachidonic acid | 5.18% |
| C20:5 ω3 | Eicosapentaenoic acid (EPA) | - |

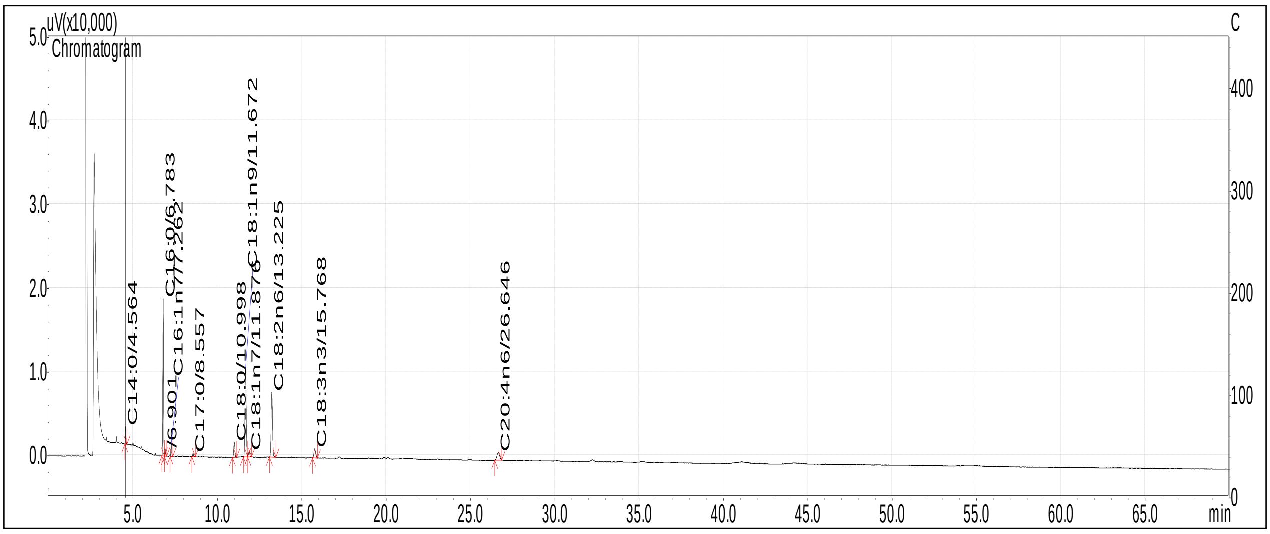


**Fig. 7:** The GC-MS chromatogram of the investigated ***C. trinoids***

## DISCUSSION

Seaweeds generate a variety of secondary metabolites that demonstrate diverse biological activities, earning them the title of biofactories. These organisms produce an array of bioactive compounds that are beneficial in different fields such as food, medicine, and agriculture[18,32]. This study covered the phytochemical characteristics of the seaweed *C. trinoids*, their manufacture of silver nanoparticles, and their potential applications as antibacterial and antioxidant agents.

Utilizing green biosynthesis methods offers several advantages compared to traditional chemical and physical manufacturing techniques. In general, green synthesis approaches are economical, easy to apply, and enable the rapid production of silver nanoparticles[33]. The eco-friendly synthesis of silver nanoparticles (AgNPs) employing algal extracts is a sustainable method that leverages the natural compounds present in seaweed extracts, such as *C. trinoids*, which can reduce silver nitrate (AgNO3) to produce AgNPs, distinguished by a characteristic brownish-yellow hue resulting from surface plasmon resonance (SPR). The synthesis process entails electrostatic interactions between silver ions and functional groups in the algal extract, such as hydroxyl and amino groups, which serve as reducing and stabilizing agents[32].

Characterization techniques such as UV-Vis spectroscopy, FTIR analysis, Transmission Electron Microscopy (TEM), and zeta potential analysis confirmed the successful production and stability of AgNPs. The zeta potential measurements indicate that the AgNPs exhibit adequate stability, which is essential for their potential use in various fields[18,34].

Numerous studies have established the evidence of algal bioactive compounds and their antimicrobial activity. Seaweeds possess various chemical compounds including phenolic compounds, flavonoids, saponins, tannins, alkaloids and polysaccharides, and distinct extracts exhibit differing biological activities[35]. These compounds significantly contribute to the synthesis of silver nanoparticles via the reduction process[36].

The evaluation of antioxidant activity for the extracts and the synthesized nanocomposites was conducted utilizing the DPPH radical scavenging assay. Prior research has demonstrated that brown seaweeds typically exhibit greater antioxidant potential in comparison to other algal varieties, a finding that aligns with their phenolic content[6,37].

The antimicrobial effectiveness of biosynthesized AgNPs has been evaluated against a range of pathogens, encompassing Gram-positive bacteria, Gram-negative bacteria, and fungi. The results indicate that the compounds possess broad-spectrum antimicrobial activity against all of the pathogens that were screened, with a particular emphasis on *Salmonella typhi*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Escherichia coli*, and *Candida albicans*[14,18]. The action of AgNPs functions through the disruption of bacterial membranes, the generation of reactive oxygen species, and the inhibition of crucial cellular functions, all of which enhance their efficacy against resistant strains[18,38].

Gas Chromatography-Mass Spectrometry (GC-MS) examination of seaweed extracts revealed a wide range of bioactive chemicals, particularly fatty acids, which are required for a variety of biological processes. Polyunsaturated fatty acids in seaweeds have been linked to a variety of health advantages, including cardiovascular protection and immune system strengthening[39]. The lipid content and fatty acid profile are affected by various factors, such as environmental conditions, species, and extraction methods[40]. The results showed that *C. trinoids* contains 10 fatty acids, with palmitic acid being the most abundant, followed by oleic acid and linoleic acid, and Vaccinic acid having the lowest amount.

## CONCLUSION

In conclusion, Seaweeds are an excellent source of bioactive chemicals with numerous biological applications. *C. trinoids* extracts were employed to synthesize silver nanoparticles using an environmentally friendly method, and illustrates that this seaweed's potential in the field of green nanotechnology. Continued investigation into the biological activity and phytochemical makeup of *C. trinoids* will increase its uses in sustainable practices, food preservation, and medicine. Further studies on the use of greenly synthesized nanomaterials are necessary to establish their viability as natural alternatives to antibiotics for treatment purposes.

**Ethical approval:**
This study received ethical approval from the Institutional Review Board of Faculty of Science, Mansoura University, ensuring that all research procedures adhered to ethical standards and guidelines.
**Conflict of interest:** There is no conflict of interest

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