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Research Article

BIOSYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES FROM MARINE MACROSCOPIC RED SEAWEED *HALYMENIA PORPHYROIDES* BOERGESEN (CRYPTON) AND ITS ANTIFUNGAL EFFICACY AGAINST DERMATOPHYTIC AND NON-DERMATOPHYTIC FUNGI

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ABSTRACT

Objective: The current study illustrates the biosynthesis of economically scalable and energy efficient colloidal silver nanoparticles (AgNPs) from marine red seaweed *Halymenia porphyroides* Boergesen (Crypton) collected from Southeast coast of Tamil Nadu, India, and their antifungal efficacy against dermatophytic and non-dermatophytic fungi was evaluated.

Methods: The biosynthesis of silver nanoparticles from marine macroscopic red seaweed *H. porphyroides* Boergesen were synthesized by green synthesis method and characterized by UV–Vis spectroscopy, Fourier transform infrared (FT-IR) spectroscopy, X-ray diffraction, thermogravimetric analysis (TGA), scanning electron microscope (SEM), and transmission electron microscopy (TEM). The efficacy of silver nanoparticles against dermatophytic and non-dermatophytic fungi was performed by disk diffusion method.

Results: The presence of silver nanoparticles with an average size between 34.3 and 80 nm and exhibiting face-centered cubic structure was confirmed. SEM revealed the morphology of the nanoparticles as spherical and TEM exhibited the nanoparticle distribution. The FT-IR spectra confirmed the presence of potential biomolecules in the seaweed crude extract which is responsible for reducing and capping the bioreduced silver nanoparticles. The UV absorption spectra at 430.5 nm revealed the characteristic spectra of the silver nanoparticles. The purity and the thermal stability of silver nanoparticles were revealed by TGA. Silver nanoparticles showed significant efficacy against dermatophytes and *Rhizopus microsporus* among non-dermatophytes.

Conclusion: Intermediate efficacy was observed against dermatophytes and among non-dermatophytic fungi *R. microsporus* exhibited better efficacy, whereas *Aspergillus flavus* were resistant to the biosynthesized silver nanoparticle.

Keywords: AgNPs, Biosynthesis, *Halymenia porphyroides*, Antifungal efficacy, Dermatophytes, non-dermatophytes.

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INTRODUCTION

Nanotechnology and nanoscience are the study of production, manipulation, and application of materials ranging in size from less than a micron to that of individual atoms [1]. The major key aspect of nanotechnology involves in the development of rapid and reliable experimental protocols such as biological and chemical processes for the synthesis of nanomaterials over wide range of chemical compositions, sizes, high monodispersity, as well as large-scale production [2]. Due to the potential for achieving specific processes and selectivity, especially in biological and pharmaceutical industries, nanomaterials and nanoparticles are considered as an important and increasing scientific research [3-8]. The increasing research and growth in the science of nanotechnology during the past few decades has opened up new avenues of fundamental and applied frontiers in the fields of materials science and engineering, such as bionanotechnology [9], quantum dots [10], surface-enhanced Raman scattering [11], and nanobiotechnology [12]. Silver nanoparticles (AgNPs), which have a high specific surface area, have been studied widely because of their unique physicochemical characteristics including catalytic activity, optical properties, electronic properties, antimicrobial activity, and magnetic properties [13-15]. Silver nanoparticles have been synthesized by various synthetic methods involving physical, chemical [16], and biochemical techniques [17] as well as biological methods using "green chemistry" protocols [18] and find their applications in food chemistry [19], biomedicine [20], and agriculture [21]. Biological/green synthesis of silver nanoparticles using microorganism [22-24], enzyme [25], plant or plant extract [26], and seaweed extract [27] has been in practice as alternatives to chemical and physical methods since they have been considered as economically stable and ecofriendly. The presence of high organic and inorganic biochemical constituents [28], phytochemicals [29], and bioactive compounds [30] in seaweeds may act as the capping/reducing agents in the biosynthesis of nanoparticles. The rise in fungal infections, especially the nosocomial infections and in patients with immunocompromised condition in cases such as cancer therapy, organ, and in HIV infections, has alarmed the researchers for an alternative treatment toward fungal pathogens [31]. One such alternative and need for novel antifungal agents are silver nanoparticles since the fungal resistance toward antibiotics has been emerging as a major threat worldwide [32,33]. Nanoparticles can be considered as potential antifungal agents [34] despite the marginal attention have been documented on the effect of silver nanoparticles as antifungal agents [35-38]. However, certain studies have been performed on the dermatophytic fungi with regard to nanoparticles [39,40]. This present investigation illustrates green synthesis of silver nanoparticles using the extract of marine macroscopic red seaweed *Halymenia porphyroides* and characterized by UV–visible spectrophotometer. Morphological and elemental analysis was carried out by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The stability of silver nanoparticles was confirmed by TGA. The possible biomolecules responsible for the reduction of the Ag+ ions and capping of the bioreduced silver nanoparticles synthesized from the experimental algae *H. porphyroides* were identified using Fourier-transform infrared (FT-IR) spectroscopy. The crystalline structure

of the silver nanoparticles, including lattice parameters, geometry, and orientation of single crystals, was studied using X-ray diffraction (XRD). Thus, algae-mediated synthesized silver nanoparticles showed intermediate zone of inhibition toward dermatophytic fungi and *Rhizopus microsporus* in non-dermatophytic fungi which showed better zone of inhibition, whereas *Aspergillus flavus* showed nil zone of inhibition which was analyzed by agar disk diffusion method.

METHODS

Collection and preparation of red seaweed extract

The marine red seaweed *H. porphyroides* Boergesen (Crypton) was collected on summer season from 2.5 m rapid intertidal regions of the Gulf of Mannar – Mandapam (latitude 9°17"N, longitude 79°11"E), Ramanathapuram district, Southeast Coast of Tamil Nadu, South India. Collected seaweed was washed with seawater for eliminating impurities such as sand, rocks, epiphytes, and epifauna. The washed samples were preserved with 5–10% formaldehyde in seawater and transported to the laboratory in a box containing slush ice. The fumes of the formaldehyde would help to fix and preserve the seaweed material. In the laboratory, the samples were washed thoroughly in running tap water to remove salt and washed 3 times using distilled water which may remove metallic compounds and it was shade dried at room temperature (37°C) for 10 days. The dried seaweed materials were crushed using mortar and pestle to get the powder form and it was stored in an air-tight container. About 1 g of crushed seaweed powder was added with 100 ml of distilled water in 250 ml conical flask and boiled for 5–10 min at 60–80°C. The crude extract was then collected and stored at 4°C for experimental use [41].

Biosynthesis and characterization of silver nanoparticles

The crude extract of the experimental marine red seaweed *H. porphyroides* Boergesen (Crypton) was used for the synthesis of silver nanoparticles. Silver nitrate (AgNO3-) (SD fine) was used for the synthesis of silver nanoparticles and double-distilled, deionized water was used for all the experiments. The silver nanoparticle formation was carried out by taking 500 mg of dry, shade dried powder samples of *H. porphyroides* in a 250 mL Erlenmeyer flask with 10^{-3} M aqueous $AgNO_{3-}$ solution and was incubated at room temperature. The pH was checked during the course of reaction and it was found to be 5.09. Nearly 95% of bioreduction of $AgNO_{3-}$ ions occurred within 24 h at stirring condition. The biosynthesis of silver nanoparticles was characterized by UV–Vis spectrophotometer (Labtron LUS-B16) in the absorbance mode and in the wavelength range between 300 to 500 nm. FT-IR spectra of biosynthesized silver nanoparticles were recorded using Thermo Scientific/Nicolet iS10 spectrometer with 1 cm^{-1} resolution in the transmission mode from wavenumbers 450–4000 cm^{-1}. The crystal structure and size of silver nanoparticles were determined using the X-ray diffractometer (Labtron LXRD-A10). Thermal stability and purity of silver nanoparticles were analyzed using TGA 4000 – PerkinElmer. A scanning electron micrograph was taken using the SEM Quanta – 400 to study the morphological characteristics of the silver nanoparticles. Further insight into morphology and the size details of the biosynthesized silver nanoparticles by the experimental algae *H. porphyroides* were investigated using high-resolution TEM (HR-TEM JEOL 3010).

Fungal susceptibility to nanosilver

Antifungal activity of the biosynthesized silver nanoparticles by the experimental algae *H. porphyroides* was tested against pathogenic fungi. The disk diffusion method for antifungal assay was adapted for testing silver nanoparticles [42]. The fungal cultures were obtained from LGC Promochem India Pvt. Ltd., Peenya, Bengaluru, India. The fungal pathogens *A. flavus* (ATCC 20048), *R. microsporus* (ATCC 22960), *Microsporum nanum* (ATCC 28951), and *Trichophyton mentagrophytes* (ATCC 28185) are the fungal strains which were used to study the antifungal efficacy of silver nanoparticles. The standard antifungal agents at the concentration of 30 µg/mL fluconazole, clotrimazole, nalidixic acid, and ketoconazole (HiMedia) were used as zone interpretive criteria. The Mueller-Hinton agar (HiMedia) supplemented with 2% glucose and 0.5 µg/L methylene blue dye medium was used for culturing the fungal stains. The Mueller-Hinton agar is readily available and shows an acceptable batch-to-batch reproducibility while the glucose provides a stable growth of most fungi and the addition of methylene blue enhances the zone edge definition [43]. The pH of the medium was maintained between 7.2 and 7.4 at 37°C. The inoculum is standardized to 0.5 McFarland using a densitometer and the plate was incubated at 35°C for 24 h. Some strains where insufficient growth has occurred after 24 h were necessary to be read after 48 h of incubation.

Statistical analysis

The data were analyzed using MS Excel 2007 and presented as mean ± SD of three replicates. One-way analysis of variance and Tukey tests were performed using "Stat plus 2009 professional" trial version software to determine significant zone differences and means were considered as statistically significant if $p<0.05$.

RESULTS AND DISCUSSION

Visual examination

The biosynthesis of silver nanoparticles was primarily identified by color change during exposure of crude seaweed extract of *H. porphyroides* into aqueous solution of silver ions which is shown in Fig. 1. The shade dried powder preparations of the experimental seaweed *H. porphyroides* were added in 10^{-3} M silver nitrate solution and allowed to react at 121°C for 20 min. The colour of the reaction solution changed to dark reddish. The control (without seaweed powder) showed no colour formation. Formation of the colour arises due to the excitation of surface plasmon vibrations where the metabolites in the seaweed extract act as the capping agent. The colour of the solution gradually intensified on heating which clearly indicates and confirms the formation of silver nanoparticles. After 24 h, there is no significant color change, indicating the saturation of the reaction of silver nanoparticle formation.

UV–visible spectroscopic analysis

The silver nanoparticles synthesized by marine red seaweed *H. porphyroides* were analyzed using UV–visible spectrophotometer (Labtron LUS-B16). The absorption spectra of silver nanoparticles formed in the reaction solution were characteristic of the nanoparticle from the seaweed and had specific absorption maxima at 430.5 nm which is shown in Fig. 2. The characteristic absorption peaks of the silver nanoparticles are in the range between 410 and 440 nm, which confirms the synthesis and formation of silver nanoparticles [44,45].

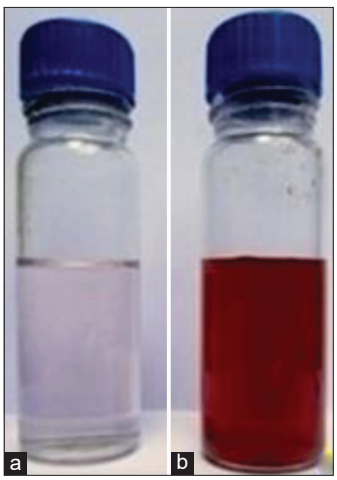

Fig. 1: Aqueous extract of *Halymenia porphyroides* before and after synthesis of silver nanoparticles. (a) Aqueous extract of algal powder before the formation of silver nanoparticles. (b) Silver nanoparticle formation in aqueous crude extract after the addition of silver nitrate

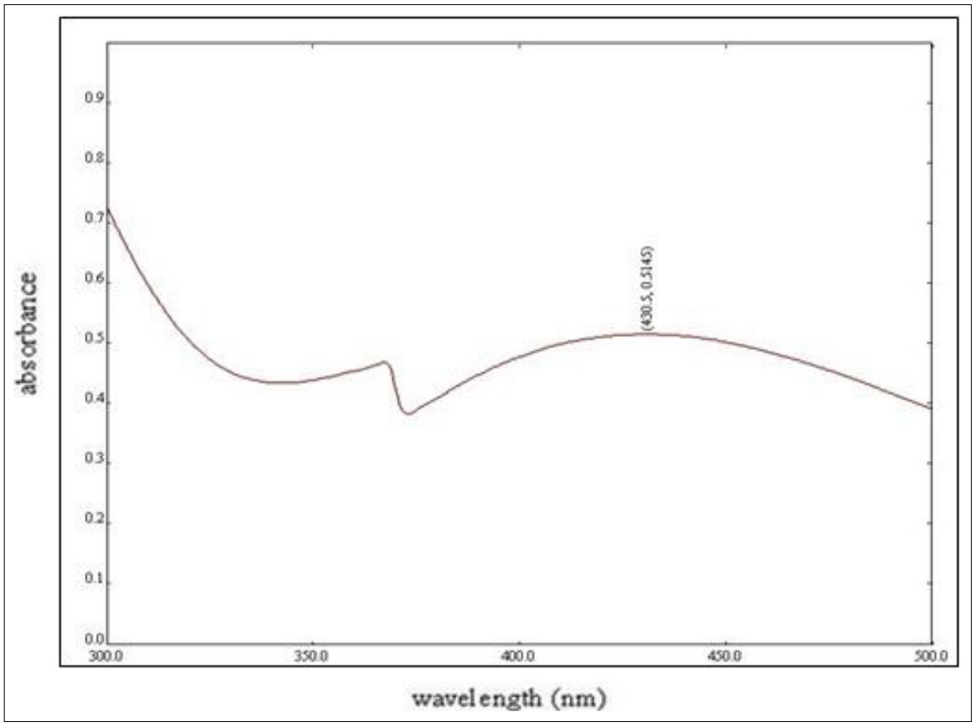

Fig. 2: UV–visible spectral analysis of silver nanoparticles biosynthesized from *Halymenia porphyroides*

The frequency and width of the surface plasmon absorption depend on the size and shape of the metal nanoparticles as well as on the dielectric constant of the metal itself and the surrounding medium [46]. The broad peaks of the absorption spectra indicate the presence of biosynthesized silver nanoparticles from the experimental seaweed *H. porphyroides* with large size distribution and are polydispersed. The interaction with the biomolecules present in the aqueous part of the reaction solution by the biosynthesized silver nanoparticles from experimental seaweed *H. porphyroides* has been indicated by UV–visible spectroscopic analysis. There were no little signs of aggregation with the biosynthesized silver nanoparticles solution which were stable for more than 6 months of observation.

FT-IR spectroscopic analysis

The FT-IR spectral measurements were carried out to identify the potential biomolecules in the crude extract of the seaweed *H. porphyroides* which is responsible for reducing and capping the bioreduced silver nanoparticles. Silver nanoparticles biosynthesized from experimental seaweed *H. porphyroides* were analyzed using FT-IR spectroscopy which is shown in Fig. 3 and Table 1. The local molecular environment of the organic molecules on the surface of the nanoparticles was determined by the IR spectra. FT-IR spectroscopy is a technique which is used to analyze the chemical composition of many organic chemicals, semiconductor materials, gases, biological samples, inorganics, and minerals. FT-IR analysis can give not only qualitative (identification) analysis of materials but, with relevant standards, also can be used for quantitative (amount) analysis. The FT-IR spectrum analysis of silver nanoparticles biosynthesized from red seaweed manifests an absorption peak at 3884.34 cm^{-1} (O–H stretch free, alcohols), 3703.80 cm^{-1} (O–H stretch free, alcohols), 3690.50 cm^{-1} (O–H stretch free, alcohols), 3662.45 cm^{-1} (O–H stretch free, alcohols), 3632.45 cm^{-1} (O–H stretch free, hydroxyl, alcohols, phenols), 3515.70 cm^{-1} (O–H stretch, H–bonded alcohol, phenols), 3393.66 cm^{-1} (O–H stretch, H–bonded, alcohols, phenols), 3367.06 cm^{-1} (O–H stretch, H–bonded, alcohols, phenols), 3343.92 cm^{-1} (O–H stretch, H–bonded, alcohols, phenols), 3276.53 cm^{-1} (N–H,H–bonded stretch, primary, secondary amines, amides), 3183.09 cm^{-1} (O–H stretch, carboxylic acids), 3117.20 cm^{-1} (O–H stretch, carboxylic acids), 3078.77 cm^{-1} (=C–H stretch, alkanes), 2976.22 cm^{-1} (C–H stretch, alkanes) [47], and 2354.71 cm^{-1} (C=N stretch, nitrile) [48]. The results revealed that the capping ligand of the silver nanoparticles may be an aromatic compound or alkanes or amines [49]. The biological molecules such as secondary metabolites could possibly play a major role in the synthesis and stabilization of the metal nanoparticles [50,51]. The Vander Waals forces between nitrogen and oxygen atoms as well as the release of protein molecules in biocompound of the experimental seaweed *H. porphyroides* may be responsible for the biosynthesis and stabilization of silver nanoparticles in an aqueous solution. These results obtained are in good agreement with the literatures [52,53].

Table 1: Fourier transform infrared spectral interpretation of silver nanoparticles biosynthesized from *Halymenia porphyroides*

Wave number (cm^{-1})	Assignments
3884.34	O–H stretch free, alcohols
3703.8	O–H stretch free, alcohols
3690.5	O–H stretch free, alcohols
3662.45	O–H stretch free, alcohols
3632.45	O–H stretch free, hydroxyl, alcohols, phenols
3515.7	O–H stretch, H–bonded alcohol, phenols
3393.66	O–H stretch, H–bonded, alcohols, phenols
3367.06	O–H stretch, H–bonded, alcohols, phenols
3343.92	O–H stretch, H–bonded, alcohols, phenols
3276.53	N–H, H–bonded stretch, 1°, 2° amines, amides
3183.09	O–H stretch, carboxylic acids
3117.2	O–H stretch, carboxylic acids
3078.77	=C–H stretch, alkanes
2976.22	C–H stretch, alkanes
2354.71	C=-N Stretch, nitrile

XRD pattern

XRD is a widely used to determine the size and crystal structure of silver nanoparticles. X-ray diffractogram of the biosynthesized silver nanoparticles by the experimental seaweed *H. porphyroides* exhibits Bragg reflection corresponding to face-centered cubic (fcc) type bulk silver. The broadened diffraction peaks around their base indicate that the silver nanoparticles are between nanosizes. XRD (Labtron LXRD-A10) analysis of biosynthesized silver nanoparticles

from *H. porphyroides* exhibited four distinct diffraction peaks is shown in Fig. 4. The XRD diffraction pattern of biosynthesized silver nanoparticles from *H. porphyroides* showed four distinct diffraction peaks at 12.13°, 28.77°, 39.87°, and 46.11° with 2^{θ} values corresponding to the lattice planes {1 0 0}, {1 1 0}, {1 1 1}, and {2 1 1} which are indexed as crystalline silver fcc phase [54]. The observed peak broadening and noise was probably related to the effect of nanosized particles and the presence of various crystalline biological macromolecules in the experimental seaweed extract. The results are in agreement with the findings of earlier investigations as reported by Paneerselvam *et al*. (2012) [55] Chandra and Kumar (2015) [56]. The diffraction patterns of silver nanoparticles biosynthesized from the experimental seaweed *H. porphyroides* indicate the uniqueness of the crystalline structure, phase purity, degree of crystallinity, and the unit cell parameters. The presence of some unassigned peaks in the X-ray diffractogram of the biosynthesized silver nanoparticles from the experimental seaweed *H. porphyroides* may be due to the biomolecules and proteins in the seaweed extract which acts as stabilizing agents [57]. The XRD pattern thus clearly shows that the silver nanoparticles are crystalline in nature.

TGA

The biosynthesized silver nanoparticles from the experimental seaweed *H. porphyroides* were subjected to TGA 4000 – PerkinElmer which is shown in Fig. 5. The thermal stability, purity, and humidity of the particles were determined using TGA [58]. The TGA relies on a high degree precision in three measurements: Weight, temperature, and temperature change. It can be applied to silver nanoparticles to determine changes in weight in relation to the changes in temperature [59]. Thermogravimetric analysis is commonly employed in research and testing to determine the characteristics of materials such as degradation temperature, absorbed moisture content of nanoparticles and polymers [60]. The silver nanoparticles biosynthesized from *H. porphyroides* showed a weight loss in a two-step process, the first step indicated a weight loss of 9% at the temperature up to 340°C which corresponds to the decomposition of bioorganic compounds present in the nanoparticle powder [61] and the second step involved weight loss of 3% at the temperature ranging from 340 to 650°C which corresponds to the decomposition of amines [62]. Thermal decomposition of the Ag^{+1} bioorganic complex at high temperature (200°C) results in an Ag atom and organic molecules [63]. The crystallite growth of silver nanoparticles biosynthesized from the experimental seaweed *H. porphyroides* appears to be consistent with "Ostwald ripening" where the stability gradient diffuses from the surfaces of small particles to the surfaces of larger particles in the Ag^{+1}-bioorganic solution [64]. The TGA results of the biosynthesized silver nanoparticles, from experimental seaweed *H. porphyroides*, show 95% purity and their stabilization was observed at 200°C which are in agreement with the findings of the earlier investigations reported by Forough and Farhadi (2010) [65] and Amjad *et al*. (2012) [66]. The 95% purity of the silver colloidal medium from the experimental seaweed *H. porphyroides* was determined by an ultrasonication method which eliminates and separates silver nanoparticles from bioorganic complexes [67].

SEM

The morphology and shape of these silver nanoparticles were carried out using SEM Quanta – 400, as shown in Fig. 6. The silver

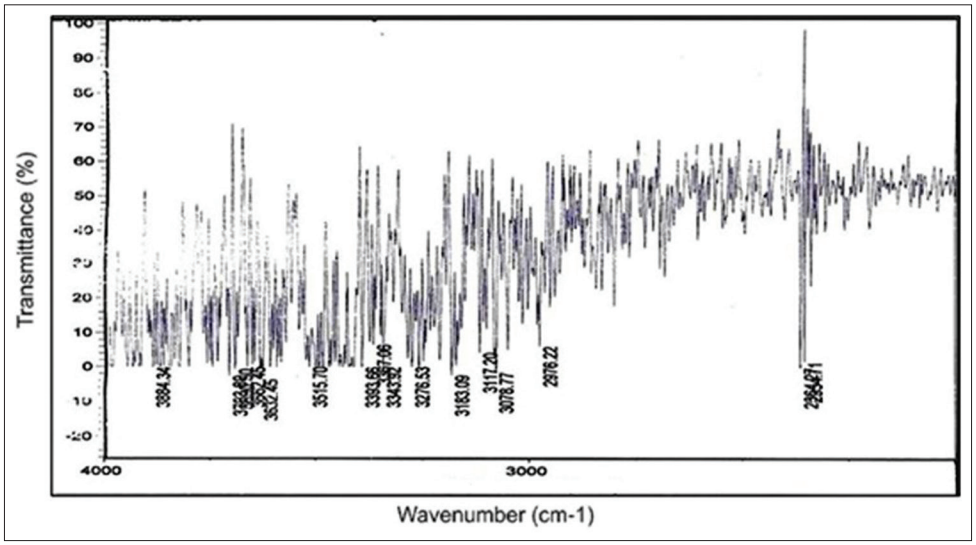

Fig. 3: Fourier transform infrared spectrum of *Halymenia porphyroides*-mediated biosynthesized silver nanoparticles

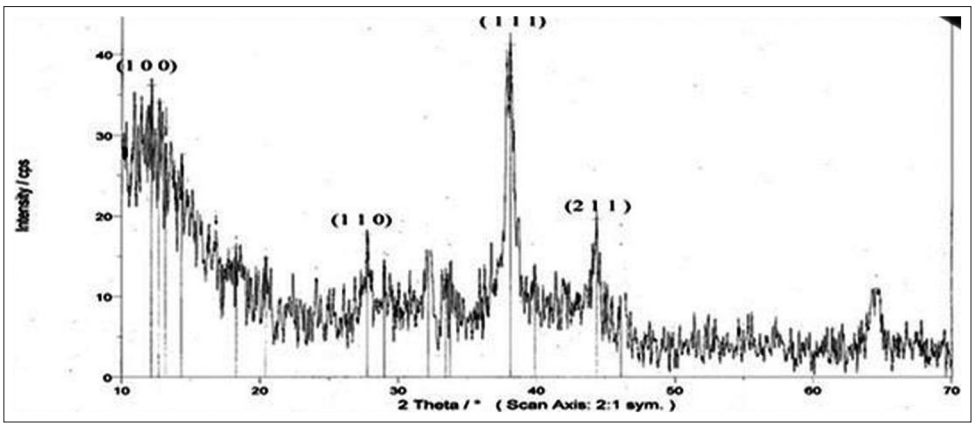

Fig. 4: X-ray diffraction analysis of sliver nanoparticles biosynthesized from *Halymenia porphyroides*

nanoparticles biosynthesized from *H. porphyroides* biomass after exposure to 10^{-3} M aqueous silver nitrate solution for 2 h showed the colloidal form of the particles in solution which microprecipitated on the surface of the biomass of the experimental seaweed. In the present study, the SEM analysis of silver nanoparticles, besides being present in colloidal form in solution, was also microprecipitated on the surface of the biomass, which were clearly indicating that the nanoparticles formed by the reduction of Ag^+ ions are bound to the surface of the cells. The silver nanoparticles biosynthesized from *H. porphyroides* formed were predominated in cubical and spherical structures with uniform shape as reported by Chandran *et al*. (2006) [68]. The brighter cubical and spherical area of the back scattered electron image corresponds to the silver nanoparticle indicating the cubic and spherical structure of silver. It is known that the shape of the metal nanoparticles has considerable changes their optical and electronic properties [69]. SEM pictures of silver nanoparticles from the experimental seaweed *H. porphyroides* showed that they were intact after the reaction and immobilization. The experimental results showed that the diameter of prepared silver nanoparticles in the solution was about 34.3–80.5 nm for *H. porphyroides*. Silver nanoparticles have a strong tendency to agglomerate which reduces the surface to volume ratio and thereby produce the catalytic effect. Therefore, a stabilizing agent is often used to prevent agglomeration. However, the agent is adsorbed on the surface of the nanoparticles, shielding them from the oxidant and reluctant and thereby inhibiting the catalysis [70].

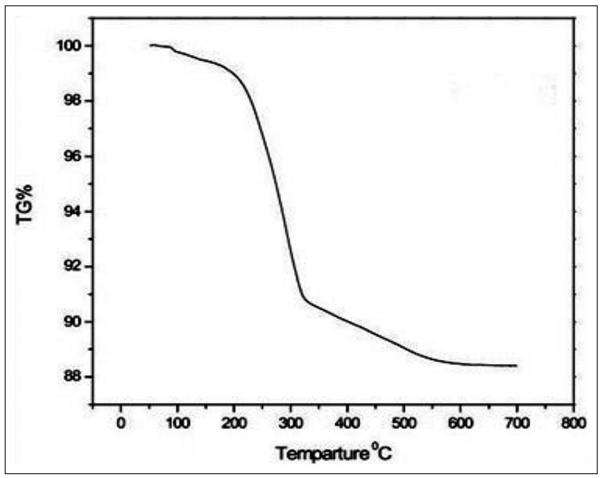

Fig. 5: Thermogravimetric analysis thermogram of silver nanoparticles biosynthesized from *Halymenia porphyroides*

TEM

The HR-TEM is the technique used to determine the size and particle distribution of silver nanoparticles [71]. The size and distribution of biosynthesized silver nanoparticles were observed by taking the micrograph from drop-coated films from silver complex solution with the extract of the experimental seaweed *H. porphyroides*. The TEM (HR-TEM JEOL 3010) images showed the formation of spherical shaped silver nanoparticles and are found in aggregates ranging from 5 to 50 nm, as shown in Fig. 7. The average mean size of silver nanoparticles from *H. porphyroides* was 32 nm. Similar results were recorded by Murugesan *et al*. (2011) [72] using red alga *Gracilaria edulis* extract as a reducing and capping agent. The shape of the metal nanoparticles has considerably changed their optical and electronic properties [73]. The HR-TEM images do not confirm the presence of capping agents on the silver nanoparticles, but under careful observation, it is noted that the silver nanoparticles are surrounded by a faint thin layer of other material, which may be the capping organic material from the experimental seaweed *H. porphyroides*. The silver nanoparticles of the TEM images are not in physical contact but are separated from each other by a uniform inter particle distance. The results of HR-TEM findings indicate that the seaweed mediated silver nanoparticle shape varies as cubical and spherical which are in agreement with earlier investigations reported by Raghunandan *et al*. (2009) [74] and Noruzi *et al*. (2011) [75].

Antifungal efficacy of silver nanoparticles

The silver nanoparticles biosynthesized from *H. porphyroides* at the concentration 30 µg/mL were tested against dermatophytes and non-dermatophytes. The dermatophytic fungi *M. nanum* (2±0.002 mm) and *T. mentagrophytes* (2±0.001 mm) showed intermediate zone of inhibition with biosynthesized silver nanoparticles from seaweed *H. porphyroides*. The non-dermatophytic fungi *R. microsporus* (4±0.002 mm) showed greater zone of inhibition with biosynthesized silver nanoparticles, whereas the non-dermatophyte *A. flavus* showed no zone formation which indicates its resistance toward biosynthesized silver nanoparticles (Fig. 8). Standard antifungal agent fluconazole exhibited higher resistance toward dermatophytic fungi *M. nanum* and *T. mentagrophytes* when compared to non-dermatophytic fungi *A. flavus* and *R. microsporus* which showed intermediate zone of inhibition. The antifungal agent clotrimazole showed an intermediate zone of inhibition for both dermatophytic and non-dermatophytic fungi and strong zone of inhibition was observed with *T. mentagrophytes,* whereas the antifungal agent ketoconazole showed least zone of inhibition toward dermatophytic and non-dermatophytic fungi. The nalidixic acid antifungal showed resistance toward dermatophytic fungi and non-dermatophytic fungi with least zone formation. The dermatophyte *T. mentagrophytes* were more susceptible to the antifungals clotrimazole and fluconazole (Fig. 8 and Table 2). The

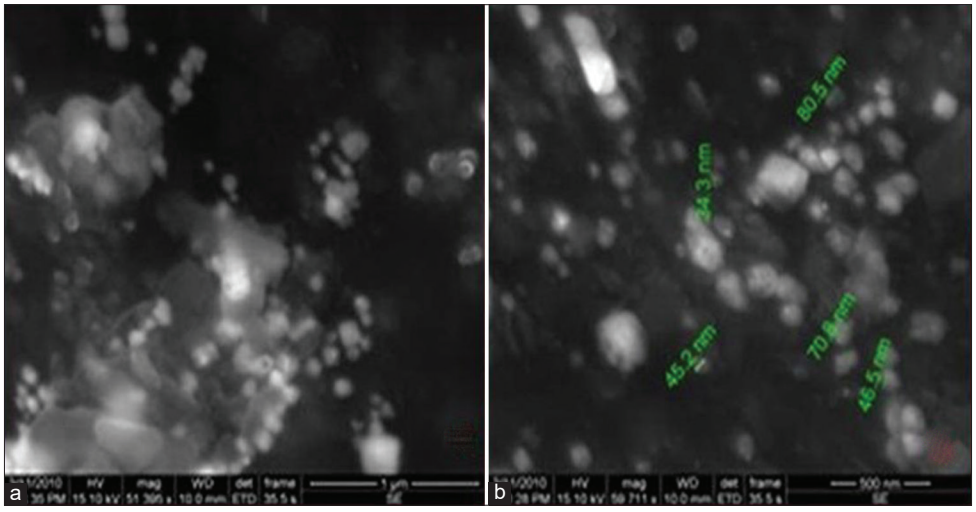

Fig. 6: Scanning electron micrograph of silver nanoparticles biosynthesized from *Halymenia porphyroides.* (a) ×51385 (b) ×59711

Table 2: Antifungal efficacy (zone of inhibition in mm) of biosynthesized silver nanoparticles from *Halymenia porphyroides*

Concentration (30 µg/mL)	*Aspergillus flavus*	*Microsporum nanum*	*Rhizopus microsporus*	*Trichophyton mentagrophytes*
Silver nanoparticles	0±0.002	2±0.002	4±0.002	2±0.001
Fluconazole	4±0.001	6±0.002	2±0.001	18±0.002
Clotrimazole	2±0.002	2±0.001	1±0.002	16±0.001
Ketoconazole	0±0.002	0±0.001	0±0.002	0±0.002
Nalidixic acid	0±0.001	0±0.001	1±0.002	1±0.001

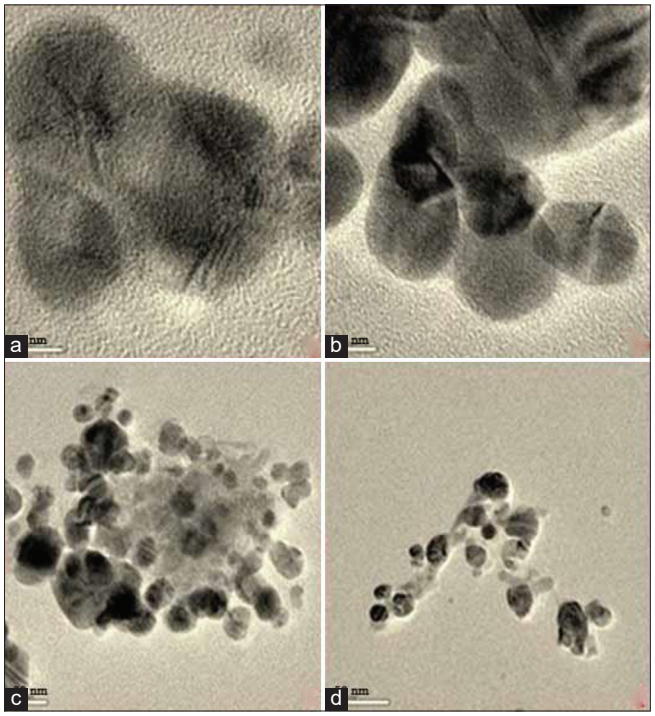

Fig. 7: (a-d) High-resolution transmission electron microscopy images of silver nanoparticles biosynthesized from *Halymenia porphyroides*

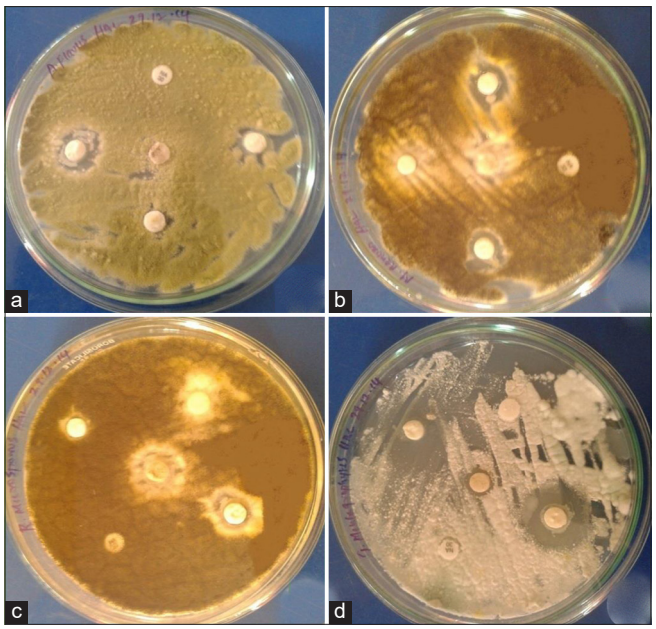

Fig. 8: Antifungal activity of biosynthesized silver nanoparticle from *Halymenia porphyroides*. (a) *Aspergillus flavus,* (b) *Microsporum nanum,* (c) *Rhizopus microsporus,* (d) *Trichophyton mentagrophytes*

adhesion and penetration of silver nanoparticles to the cell membrane of the fungal pathogen thereby producing a with less molecular weight at the center of the fungi resulting in the attachment of nanosilver to the respiratory sequence and consequently stopping the cell division leading to the death of fungal cells might be the mechanism involved in the efficacy pathway of the silver nanoparticles [76]. The size and the surface are of the silver nanoparticles largely determine the formation of pits in the fungal cell wall [77]. Disrupting the membrane potential of the cell membrane of the fungal pathogen, leading to the cell death, might be the other possible mechanism [78-81]. The antimicrobial and antifungal properties of biosynthesized sliver nanoparticles from marine seaweed extract were confirmed from the current study on antifungal efficacy [82,83].

CONCLUSION

The results from the present investigation have concluded that the silver nanoparticles biosynthesized from marine seaweed *H. porphyroides* showed intermediate effect against dermatophytes, whereas among non-dermatophytic fungi, especially *R. microsporus,* exhibited better efficacy and on the other non-dermatophyte *A. flavus* were resistant to the biosynthesized silver nanoparticles. As elaborated earlier not much work has been documented in the efficacy of silver nanoparticles toward fungal pathogens, whereas this current study may pave a pathway for the use of colloidal silver in the antibiotic or antifungal resistance strains, especially will be helpful in treating immunocompromised patients and in nosocomial infections. The results also reveal that the dermatophytes are susceptible to silver nanoparticles with intermediate, whereas the non-dermatophytic fungi *R. microsporus* exhibited much better zone of inhibition toward the algal-meditated silver nanoparticles when compared to standard antifungal agents used in the study. This study forms the basis of the exoskeleton for more research with silver nanoparticles toward fungal pathogens and drug-resistant strains.

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AUTHORS' CONTRIBUTIONS

Dr. Vishnu Kiran Manam designed and performed the work, whereas Dr. Murugesan Subbaiah suggested the relevant changes during the course of the work and the proofreading of the manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHORS' FUNDING

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