**International Journal of Botany Studies**
**ISSN: 2455-541X; Impact Factor: RJIF 5.12**
**Received: 03-02-2021; Accepted: 12-03-2021: Published: 20-03-2021**
**www.botanyjournals.com**
**Volume 6; Issue 2; 2021; Page No. 243-248**

# Green synthesis of iron nanoparticles using aqueous extract of *Turbinaria Conoides* (J. Agardh) and their anticancer properties

**Asha DV Bensy, G Johnsi Christobel***
Department of Botany and Research Centre, Nesamony Memorial Christian College, Affiliated to Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli, Tamil Nadu, India

**Abstract**
Marine macroalgae produce numerous bioactive compounds with potential pharmacological properties. In this study, macroalga was collected from Gulf of Mannar, India and identified as, *Turbinaria conoides* (J. Agardh). The aqueous extract of *T. conoides* was used to synthesize iron nanoparticles (NPs). The synthesized iron NPs were characterized by X –ray diffraction analysis, Scanning Electron Microscopy, and Transmission Electron Microscopy. The synthesized NPs showed potent activity against DLD1 and HeLa cell lines.

**Keywords:** seaweed, phytochemicals, phenols, antioxidant

## Introduction
Seaweeds are considered as the source of various bioactive compounds as they are able to synthesize potential secondary metabolites (Smit, 2004) [16] characterized by various biological activities including, antibacterial, antiviral and anticancer activities (Chakraborthy *et al*., 2010) [5]. Anti tumour and antifungal activities were detected in red, brown and green algae. The antimicrobial property of seaweed mainly based on algal species, extraction method and solvents used for extraction. However, maximum bioactive potential was obtained from the dried macroalgae samples than fresh algal samples and have been reported previously by various research groups (Manivannan *et al*., 2011) [11]. Seaweeds contain a wide variety of numerous bioactive compounds offering a potential source of novel drugs with very low toxicity (Ganesan *et al*., 2020) [7]. Sample preparation, extraction method and processing greatly affected the bioactivity of macroalgae (Moraes-de-Souza *et al*., 2008) [12]. Many findings showed that a rich of dietary intake of various natural phenols with the presence of various types of antioxidants such as, phenols and flavonoids generally found in seaweeds and plants is mainly associated with reduced risk of developing chronic diseases, longer life expectancy, and various types of cancer. The present investigation aimed to analyze *in vitro* antioxidant, antibacterial activity and characterization of iron oxide nanoparticles using *Turbinaria conoides* (J. Agardh) collected from Gulf of Mannar, Tamilnadu, India.

## Materials and Methods
### Collection of seaweed
The macro algae samples (*Ulva faciata* Delile, *Caulerpa peltata* J.V. Lamour., *Paduba oavibuca* (L.) Thivy, *Champia compressa* Harv, and *Turbinaria conoides* (J. Agardh) were collected during the study period (July 2018 – December 2018) by manually and detaching a portion from the sea bed, exposed rock surfaces of rocky coast in Gulf of Mannar, Tamilnadu, India. Macro algae were cleaned thoroughly to remove debris. All the collected macro algae were photographed in fresh condition.

### Identification and Selection of Algae
Identification of algae was carried out using the manual authenticated by Botanical Survey of India (BSI/SRC/5/23/2020/Tech/885). Selection of algae was mainly based on availability and previous literature on phytochemical components of algae. The selected algae was identified as *Turbinaria conoides* (J. Agardh).

### Preparation of Seaweed Extract
10 g air dried powder was weighed and transferred in 100 ml solvent such as, methanol, chloroform, acetone, ethyl acetate and ethanol for 24 h. The Erlenmeyer flask was kept on an orbital shaker at 150 – 200 rpm for 24 h. Then the sample was filtered using a Whatman's no 1 filter paper. The filtrates were further evaporated under reduced pressure and semi-solid material was obtained with the help of rotary evaporator. The dried residue was further stored in a plastic vials. The extraction procedure was repeated three times.

### Green Synthesis of Iron Nanoparticles
100 g fresh seaweed was ground using a pestle and mortar and microwave assisted extraction was performed using a microwave oven. The extract was filtered and stored at 4 ºC and used for the green synthesis of Fe NPs. Ferrous sulphate was prepared at 1 mm concentration and aqueous extract of was added in drop wise manner with continuous stirring. Reduction reaction was stopped after brownish black colour formation and it was incubated at 32 ± 2 °C for 4 h. Iron

oxide NPs was then isolated from the solution by evaporating water on a hot plate dried for overnight using a dryer. The synthesized NPs were purified by refluxing using Milli Q water followed by absolute ethanol.

### Characterization of NPs

The colour change of $FeCl_3$solution (100 mL) (1 mM) after the addition of alga extract (1 m) from yellowish-brown to black was confirmed the synthesis of iron oxide NPs. It was dispersed in distilled water and the spectrum was analyzed between of 200-800 nm using a UV-visible spectrophotometer. X –ray diffraction of iron oxide NPs was performed using Philips X'Pert Pro instrument. The iron NPs was scanned within the 2 Theta range of 20–80 Theta angle. The surface structure was characterized using SEM JEOL-MODEL 6390 wafter coating on copper grid for 5 min on mercury lamp. The shape and size of the iron NPs was evaluated by Transmission Electron Microscopy (TEM).

### Anticancer Activity Analysis

Green synthesized NPs were used for the determination of anticancer activity. DLD-1(Human Colorectal Adenocarcinoma) cells and HeLa (cervical cancer cells) were initially procured from National Centre for Cell Sciences (NCCS), Pune, India. It was maintained in Dulbecco's modified Eagles medium (DMEM). Then the cells were cultured in 25 $cm^2$ tissue culture flask with DMEM supplemented with sodium bicarbonate, L-glutamine and 10% FBS (Merck, Germany) and antibiotics. Cell lines were kept in a humidified $CO_2$ incubator at 37 ºC. After 24 h the tissue culture medium was removed and NPs were added at various concentrations (100µg, 50µg, 25µg, 12.5µg, 6.25µg in 500µl of DMEM). It was further incubated in a humidified 5% $CO_2$ incubator at 37 ºC. The morphological changes were detected using an inverted phase contrast tissue culture microscope. The percentage viability was calculated using the following formula.

$$\% \text{ of viability} = \frac{\text{Mean OD Samples x } 100}{\text{Mean OD of control group}}$$

## Results and Discussion

### *T. Conoides* Extract and Biological Activity

Marine macroalgae produce various bioactive compounds with potential pharmacological properties. These organisms produce various compounds in response to the characteristics as well as environmental condition. In this study, a potential macroalga was collected from Gulf of Mannar, India and identified as, *T. conoides* (J. Agardh) (Fig. 1b). Seaweeds have been widely recognized as potential producers of an enormous range of compounds with bioactive potential compounds. However the activity of the same genus or species could vary depending on the geographical locations due to seasonal and environmental parameters (Chakraborthy *et al*., 2010) [5].

**Fig 1:** Appearance of *T. conoides* collected from Gulf of Mannar, Tamilnadu India.

### Characterization of Iron NPs

An absorption range from 200 nm (near the Ultra violet) to 800 nm (in the very near to infra-red) was clearly observed in the sample. In Figure 2, the peaks are high between 200 and 300 nm indicated the reduction of iron oxide. X-ray diffraction analysis provides valuable information on crystalline structure, average grain size, preferred crystal orientations, phases and crystal defects. The present finding revealed that *T. conoides* showed the wide intensities for the determination of atomic positions in the lattice (Figure 3). XRD peaks are generally obtained by constructive interference of a monochromatic beam of X-rays scattered at specific angles from each set of lattice planes in a sample. Generally, online search tool for X-ray powder diffraction patterns enables quick phase identification for the identification of various samples. Figure 4 clearly revealed that the presence of green synthesized iron NPs in the sample. The SEM images made possible the visualization of the morphology of the NPs and to determine the size of NPs. TEM analysis clearly revealed the presence of NPs with 27.59 nm size (Figure 5). Macro algae are important sources of phytochemicals involved in the biosynthesis of types of metallic NPs. Macro algae such as, *Stoechospermum marginatum*, *Laminaria japonica*, *Turbinaria conoides*, and *Sargassum wightii* (Singaravelu *et al*., 2007; Ghodake and Lee, 2011; Rajathi *et al*., 2012) [15, 8, 13]. Green synthesis of iron NPs was initially characterized by colour formation from yellowish-brown to black in the mixture and showed surface plasmon resonance band was centered at 240 – 320 nm showed the presence of iron NPs. SEM and TEM analysis revealed the morphology and crystalline structure of green synthesized NPs and the NPs size range of 27 to 30 nm. Recently, El-Kassas *et al*. (2016) [6] used *Sargassum acinarium* (Linnaeus) and *Padina pavonica* (Linnaeus) for the biosynthesis of iron oxide NPs.

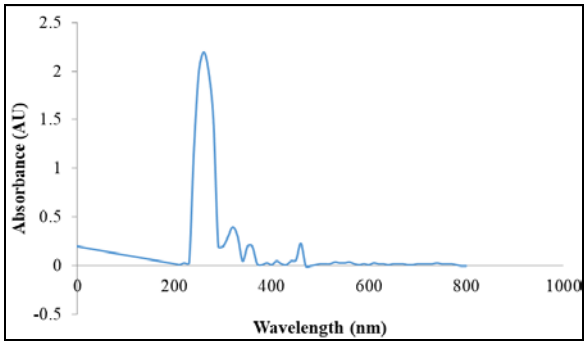



**Fig 2:** UV spectroscopy analysis of green synthesized iron NPs using *T. conoides* extract

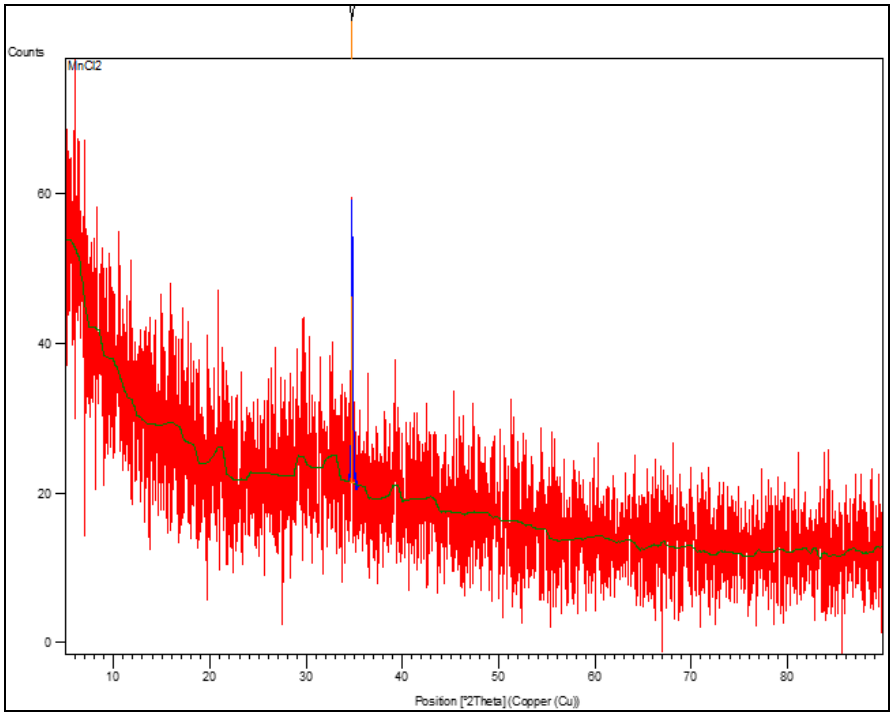



**Fig 3:** XRD of iron NPs green synthesized using *T. conoides* extract

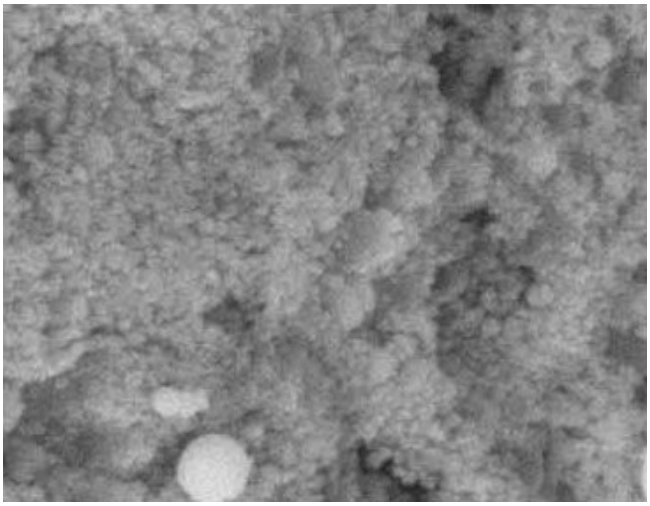


**Fig 4:** Scanning Electron Microscopy analysis of iron NPs green synthesized using *T. conoides* extract.

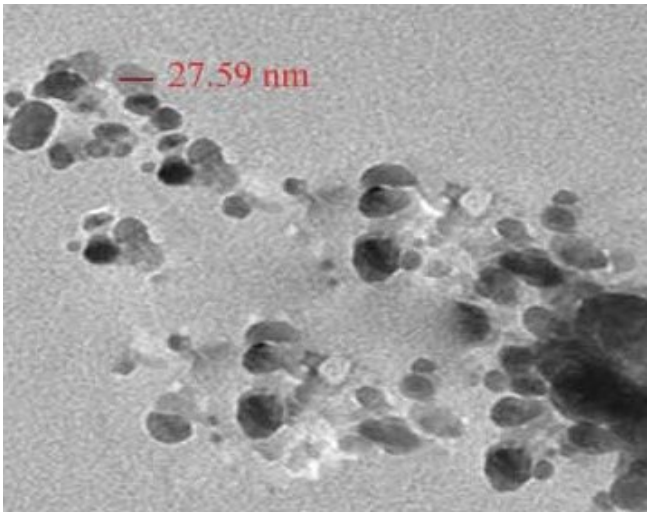



**Fig 5:** Transmission Electron Microscopy image of iron NPs green synthesized using *T. conoides*

**Anticancer Activity of Iron NPs**

MTT assay is frequently used to evaluate the NPs to determine proliferative or cell toxicity activity. The LC-50 value was found to be 157.366 µg/mL and results indicated that NPs had significant activity against HeLa and DLD1 cell lines and the results were depicted in Table 1. Anticancer activity of iron NPs synthesized using *T. conoides* against HeLa and DLD1cell lines was described in Fig. 7 and 8. Any new drug required cytotoxic analysis in cancer cell and cell viability test is determined under microscope (Arasu *et al*., 2019) [1, 2]. In MTT assay, the linear relationship between colour produced and metabolic process of active cells and useful for the determination of the rate of proliferation or cell death. SEM and TEM analysis was employed to study the morphological and structural features of synthesized FeNPs. SEM and TEM analysis revealed uniform, spherical shaped NPs within 30 nm size. In this study, some of the larger particles were identified due to aggregation of NPs because of evaporation of alcohol during the preparation of samples. This kind of NPs aggregation has been reported previously. XRD analysis clearly indicated the presence of metallic silver nanocrystals due to surface plasmon resonance. The intense signals between 2 and 4 KeV indicated the presence of metallic nanocrystals (Singaravelu *et al*., 2007) [15]. The synthesized nanoparticles have the ability to inhibit the growth of cell lines HeLa and DlD1. The anticancer activity of nanoparticles has been reported previously (Valsalam *et al*., 2019; Arasu *et al*., 2019; Venkatadri *et al*., 2020; Lydia *et al*., 2020; Malar *et al*., 2020) [17, 1, 2, 18, 9, 10].

**Table 1:** Viability of HeLa and DLD1 cell lines treated with iron NPs at various concentrations.

| Sample Concentration (µg/mL) | Viability (%) | |
|---|---|---|
| | HeLa | DLD1 |
| **Control** | 100 | 100 |
| 6.25 | 90.35 | 91.22 |
| 12.5 | 85.44 | 79.07 |
| 25 | 79.49 | 68.82 |
| 50 | 51.35 | 60.21 |
| 100 | 26.41 | 27.28 |

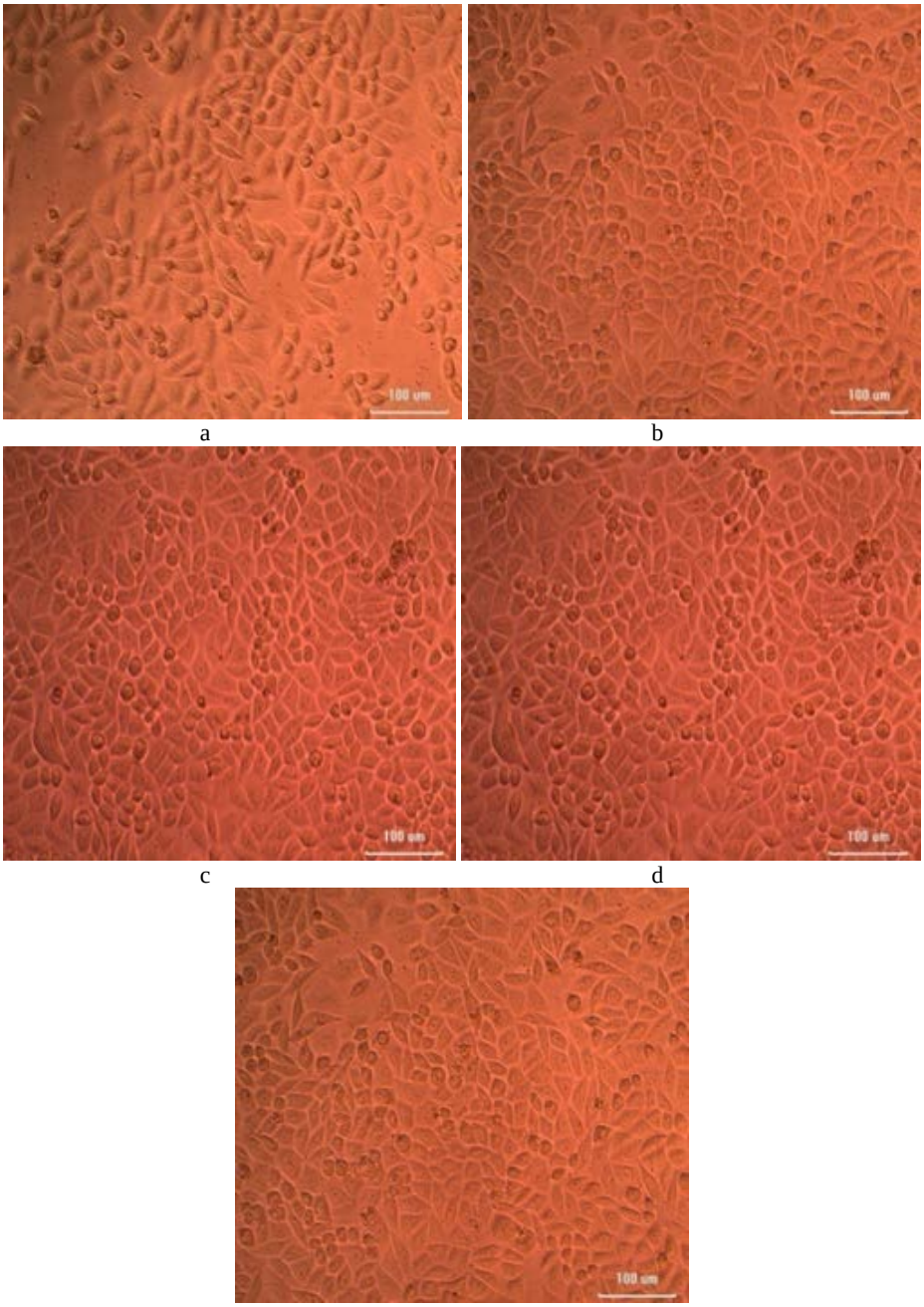
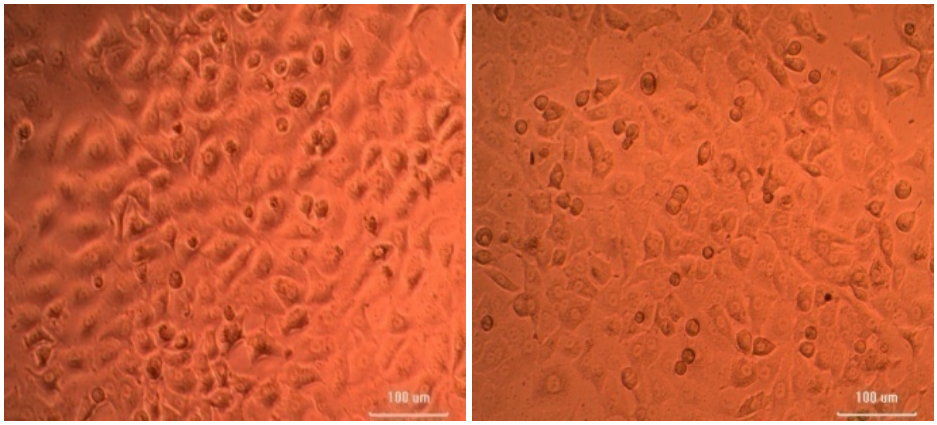



**Fig 6:** Anticancer activity of iron NPs synthesized using *T. conoides* against HeLa cell lines.



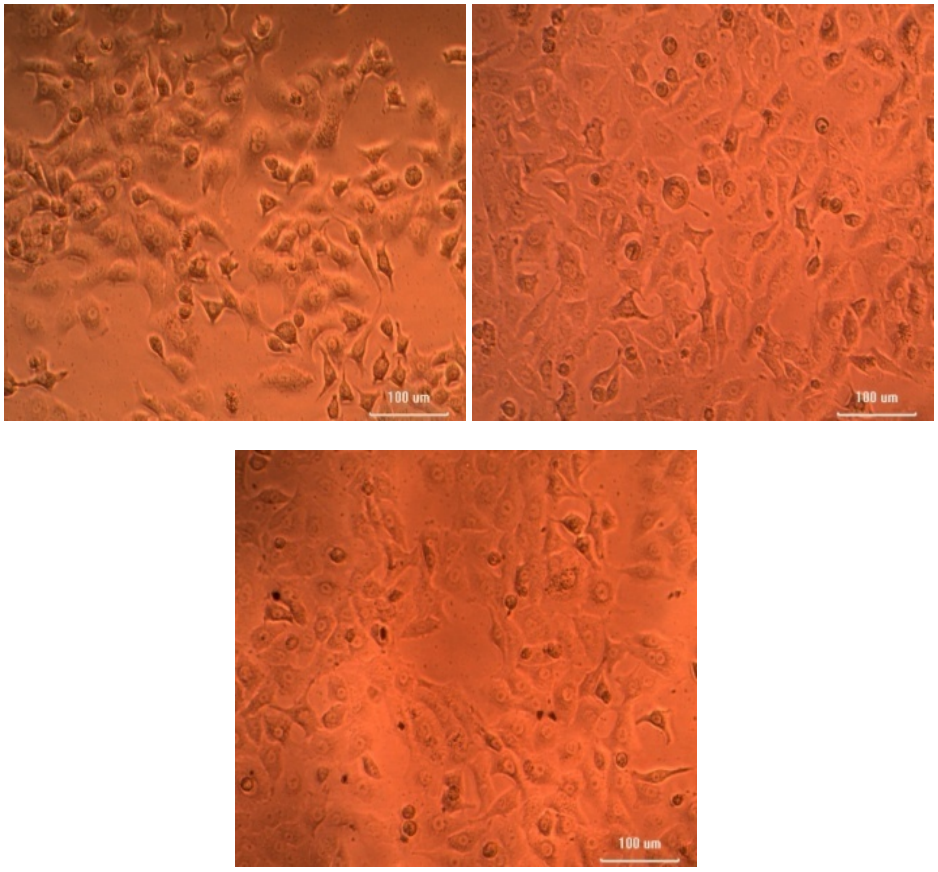



**Fig 7:** Anticancer activity of green synthesized iron NPs against DLD1cell lines.

## Conclusion

The aqueous extract of *T. conoides* was used to synthesize iron NPs. The present finding eliminates the wide use of various chemical substances as stabilizing and reducing agent. Because sea weed has various chemical constituents which are polyphenolic substances and fucoidan, it shows a dual application as both stabilizing and reducing agent for iron NPs. The synthesized NPs showed potent activity against DLD1 and HeLa cell lines.

## References

1. Arasu MV, Arokiyaraj S, Viayaraghavan P, Kumar TSJ, Duraipandiyan V, Al-Dhabi NA *et al*. One step green synthesis of larvicidal, and azo dye degrading antibacterial nanoparticles by response surface methodology. Journal of Photochemistry and Photobiology B: Biology. 2019; 190:154-162. https://doi.org/10.1016/j.jphotobiol.2018.11.020
2. Arasu MV, Madankumar A, Theerthagiri J, Salla S, Prabu S, Kim HS *et al*. Synthesis and characterization of ZnO nanoflakes anchored carbon nanoplates for antioxidant and anticancer activity in MCF7 cell lines. Materials Science and Engineering. 2019; C102:536-540. https://doi.org/10.1016/j.msec.2019.04.068
3. Bindhu MR, Umadevi M, Esmail GA, Al-Dhabi NA, Arasu MV. Green synthesis and characterization of silver nanoparticles from Moringa oleifera flower and assessment of antimicrobial and sensing properties. Journal of Photochemistry and Photobiology B: Biology. 2020; 205:111-836. https://doi.org/10.1016/j.jphotobiol.2020.111836
4. Campos-Takaki GM, Diu MBS, Koening ML, Pereira EC. Screening of marine algae from Brazilian northeastern coast for antimicrobial activity. Botanica Marina. 1988; 31:375-377. https://doi.org/10.1515/botm.1988.31.5.375
5. Chakraborthy K, Lipton AP, Paulraj R, Vijayan KK. Antibacterial diterpernoids of *Ulvafasciata* Delile from South-western coast of Indian Peninsula. Food Chemistry. 2010; 119:1399-1408. https://doi.org/10.1016/j.foodchem.2009.09.019
6. El-Kassas HY, Aly-Eldeen MA, Gharib SM. Green synthesis of iron oxide (Fe3O4) nanoparticles using two selected brown seaweeds: characterization and application for lead bioremediation. Acta Oceanologica Sinica. 2016; 35(8):89-98.
7. Ganesan AR, Mohanram MSG, Balasubramanian B, Kim IH, Seedevi P, Mohan K. *et al*. Marine invertebrates' proteins: A recent update on functional property. Journal of King Saud University-Science. 2020; 32(2):1496-1502. https://doi.org/10.1016/j.jksus.2019.12.003
8. Ghodake G, Lee DS. Biological synthesis of gold nanoparticles using the aqueous extract of the brown algae *Laminaria japonica*. Journal of Nanoelectronics and Optoelectronics. 2011; 6:1-4. https://doi.org/10.1166/jno.2011.1166
9. Lydia DE, Khusro A, Immanuel P, Esmail GA, Al-Dhabi NA, Arasu MV *et al*. Photo-activated synthesis and characterization of gold nanoparticles from *Punica granatum* L. seed oil: An assessment on antioxidant and anticancer properties for functional yoghurt nutraceuticals. Journal of Photochemistry and Photobiology B: Biology. 2020; 206:111-868. https://doi.org/10.1016/j.jphotobiol.2020.111868

10. Malar TJ, Antonyswamy J, Vijayaraghavan P, Kim YO, Al-Ghamdi AA, Elshikh MS, Hatamleh AA *et al*. *In-vitro* phytochemical and pharmacological bio-efficacy studies on *Azadirachta indica* A. Juss and Melia azedarach Linn for anticancer activity. Saudi Journal of Biological Sciences. 2020; 27(2):682-688. https://doi.org/10.1016/j.sjbs.2019.11.024
11. Manivannan K, Karthikaidevi G, Anantharaman P, Balasubramanian T. Antimicrobial potential of selected brown seaweeds from Vedalai coastal waters, Gulf of Mannar. Asian Pacific Journal of Tropical Biomedicine. 2011; 1:114-120. https://doi.org/10.1016/S2221-1691(11)60007-5
12. Moraes-de-Souza RA, Oldoni TLC, Regitano-d'Acre MAB, Alencar SM. Antioxidant activity and phenolic composition of herbal infusions consumed in Brazil. CYTA Journal of Food. 2008; 6(1):41-47. https://doi.org/10.1080/11358120809487626
13. Rajathi FAA, Parthiban C, Ganesh Kumar V, Anantharaman P. Biosynthesis of antibacterial gold nanoparticles using brown alga, *Stoechospermum marginatum* (kützing). Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2012; 99:166-173. https://doi.org/10.1016/j.saa.2012.08.081
14. Salvador N, Gómez Garreta A, Lavelli L, Ribera MA. Antimicrobial activity of Iberian macroalgae. Scientia Marina. 2007; 71:101-114. https://doi.org/10.3989/scimar.2007.71n1101
15. Singaravelu G, Arockiyamari J, Ganesh Kumar V, Govindaraju K. A novel extracellular biosynthesis of monodisperse gold nanoparticles using marine algae, *Sargassum wightii* Greville. Colloids and Surfaces B: Biointerfaces. 2007; 57:97-101. https://doi.org/10.1016/j.colsurfb.2007.01.010
16. Smit AJ. Medicinal and pharmaceutical uses of seaweed natural products. A review. Journal of Applied and Phycology. 2004; 16:245-262. https://doi.org/10.1023/B:JAPH.0000047783.36600.ef
17. Valsalam S, Agastian P, Arasu MV, Al-Dhabi NA, Ghilan AKM, Kaviyarasu K *et al*. Rapid biosynthesis and characterization of silver nanoparticles from the leaf extract of Tropaeolum majus L. and its enhanced in-vitro antibacterial, antifungal, antioxidant and anticancer properties. Journal of Photochemistry and Photobiology B: Biology. 2019; 191:65-74. https://doi.org/10.1016/j.jphotobiol.2018.12.010
18. Venkatadri B, Shanparvish E, Ramesh kumar MR, Arasu MV, Al-Dhabi NA, Ponnusamy VK *et al*. Green synthesis of silver nanoparticles using aqueous rhizome extract of *Zingiber officinale* and *Curcuma longa*: In-vitro anti-cancer potential on human colon carcinoma HT-29 cells. Saudi Journal of Biological Sciences. 2020; 27(11):2980-2986. https://doi.org/10.1016 /j.sjbs.2020.09.021