INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 16, No. 4, p. 404-417, 2020

RESEARCH PAPER OPEN ACCESS

Brown seaweed *Sargassum polycystum* (C. Agardh) extract as mediator in the green synthesis of colloidal gold nanoparticles from Malita, Davao Occidental Philippines

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Key words: Brown Seaweed, *Sargassum polycystum*, Reducing agent, Green synthesis, Gold nanoparticles

http://dx.doi.org/10.12692/ijb/16.4.404-417 Article published on April 30, 2020

Abstract

One of the paradigm shifts in the field of nanotechnology is the development of environmentally friendly, biocompatibility and green approaches in the synthesis of metal nanoparticles. In this study, the extracts of Brown seaweed, *Sargassum polycystum* (C. Agardh) were utilized as reducing agents in the green synthesis of gold nanoparticles. The produced gold nanoparticles were characterized using UV-visible spectroscopy, Fourier Transform infrared (FTIR) spectroscopy, Scanning electron microscopy (SEM) and Energy dispersive X-ray (EDS) spectroscopy. The different volumes of brown seaweed extract namely 25, 20, 15, 10 and 5ml when added with Tetrachloroauric acid gradually changed from color brown to ruby red, a characteristics reaction of gold nanoparticles. UV-vis spectral analysis of the gold nanoparticles showed strong peaks around 526nm to 548nm, an optical characteristic of gold nanoparticles. The presence of elemental gold was also confirmed by the EDS analysis. The SEM image of the gold nanoparticles clusters of spherical morphology with a size range of 68.5 to 240nm. The FTIR spectra of the brown seaweed mediated synthesized gold nanoparticles showed strong peaks corresponding to hydroxyl groups, and primary amines functionalities which probably acted as reducing agent, stabilizing agent and capping agent during the synthesis of gold nanoparticles.

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Introduction

One of the paradigm shifts in the field of nanotechnology is the development of environmentally friendly, cheaper and green approach in the synthesis of metal nanoparticles. In the synthesis of nanoparticles, the method must exhibit rapid chemical reaction, highly stable and has better uptake on Biomolecules (Dhas, TS. 2012).

One of the most explored materials is the gold nanoparticles which are known with its excellent optical, electronic, and magnetic properties, biocompatibility and non-toxic which are highly desirable for biomedical applications (Khanna *et al.*, 2019). Thus, gold nanoparticles are developed for applications such as bio-sensor probes, bioremediation, diagnostics, electronic conductors, therapeutic agents, organic photovoltaics, drug delivery in biological and medical applications and catalysis (Khanna P. 2019).

Currently, gold nanoparticles are fabricated by physico-chemical synthesis such as the Turkevich, Brust, seeded growth, and miscellaneous methods. The physico-chemical methods generate efficient gold nanoparticles but they employ various synthetic chemicals which are used for reducing, capping, and stabilizing agents which generate waste products that causes damage when released in the environment during large scale production. Furthermore, the use of toxic chemicals and solvents in the methods may also be problematic for downstream biological applications (Arunkumar L, 2019).

To mitigate the problem, alternative methods of gold nanoparticles synthesis have been proposed. A process that do not employ toxic chemicals and adhere on the principles of green chemistry, which advocate the utilization of rapidly biodegradable reagents, limiting waste products, synthesis at ambient temperature and pressure and the minimization of detrimental environmental impact. The method of green synthesis of gold nanoparticles utilizes biological components like carbohydrates, lipids, nucleic acids, and proteins produced in nature (Khanna P. 2019).The method of green synthesis is fundamentally clean, ecofriendly and low cost of production.

Green synthesis was demonstrated in microbes like bacteria, fungi, actinomycetes; biomolecules, plant extracts and macro-algae to mediate the production of gold nanoparticles (Kharissova *et al.*, 2013). Seaweeds were considered as an attractive source due to its excellent properties for metal uptake, faster doubling time, easily scalable and well developed systems, cells can be readily disrupted, easily harvested, low cost in large-scale synthesis, the nucleation and crystal growth are accelerated due to the presence of negative charge on the surface of the cell (Khanna P. 2019).

The mechanism behind the green synthesis of seaweeds is due to the abundant functional groups are present in the cell wall namely aromatic nitro compound, amides, alkyl chloride, organophosphorus compounds, amines, sulfonyl chloride, primary amines, ketones, phosphines and aliphatic compound indicated the potential sources of compounds that will act as reducing, capping and stabilizing agents for the successful synthesis of gold nanoparticles (Kharissova *et al.*, 2013, Kaewsarn *et al.*, 2002).

Moreover, the presence of fucoxanthin, a xanthophyll pigment, the main component responsible for the colour of the brown seaweeds. Fucoxanthin has been known for its potential on reduction of metal ions to form gold nanoparticles (Mittal *et al.*, 2017). Brown algae possess bioactive compounds like polyphenols, terpenoids, flavones, amines, protein and enzymes can potentially play the role of reducing agents, stabilizing agents and capping agents during the synthesis of gold nanoparticles (Khanna P. 2019).

In the selected coastal areas of Davao Occidental such as Malita, Don Marcelino and Jose Abad Santos, brown seaweeds such as *Turbinaria ornata*, *Turbinaria conoides, Sargassum polycystum, Padina australis,* and *Padina minor* are highly abundant (Sarda A. 2018, Balawag B. 2016 and Tulo, 2018). So far, to the best knowledge of the researcher, *Sargassum crassifolium* was the only brown seaweeds is tested to mediate synthesis of gold nanoparticles in the Philippines (Ouano *et al.*, 2018). Among the brown seaweeds, Sargassum species is

reported to possess higher content of alginic acid (Jerod *et al*., 2015) fucoidans (Sinurat *et al*., 2016) and phytochemicals (Balanquit *et al*., 2015).

Previously, the thallus (Kanimozhi *et al*., 2015) and leaves (Sivaraj *et al*., 2015) of the *S. polycystum* were used as the source of the extract for synthesis of silver and spherical gold nanoparticles. In the current work, the whole plant S. *polycystum* was used as the source of the extract for rapid synthesis of monodispersed spherical gold nanoparticles at room temperature with no further optimization.

Materials and Methods

Preparation of Chemicals

The experiment used the 1 milli molar (mM) of Tetrachloroauric (III) acid trihydrate as the precursor of the synthesis of Gold nanoparticles. To ensure reproducibility of the results, all the glasswares used in the study was cleansed using the Aqua regia.

Sample Collection of Brown Seaweed

A preliminary survey was conducted by looking to previous studies conducted and an actual site visit by the researcher to determine the most abundant brown seaweed of the sampling area. Random collections of *Sargassum polycystum* were done during the month of April-July, 2019. A slate board and pencil was used in noting down information and other data gathered during sampling. The brown seaweed was collected by manually picking from the Intertidal area of Tubalan, Malita Davao Occidental, Philippines ("6 ° 30' 50" N, 125° 34' 41" E"). The collected brown algae species were identified by using the field guide and atlas of the Seaweed resources of the Philippines (Gavino, 1997) and Field guide to common mangroves, Sea grasses and Algae of the Philippines (Calumpong, 1997).

Preparation of brown Algal Extract

The collected brown algae were washed thoroughly with 1 liter of tap water to remove epiphytes that may have lived in the fronds, blade and thallus. The clean seaweeds were washed subsequently with distilled water for several days and air dried for 3-5 days and oven dried at 60° Celcius for 1 day. The dried seaweeds were ground into fine powder using an electric blender. Using the 500 microns sieve, the seaweed powder was segregated. The 5 grams of brown seaweed powder were suspended in 100ml of deionized water in a 300ml Erlenmeyer flask followed by boiling for 15 minutes. The extract obtained was filtered through Whatman filter paper No.1 (50 mm; Sigma, Bangalore, India). The filtered extract was centrifuged at 5,000rpm for 10 minutes and the supernatant was used both as a reducing agent, capping agent and as a stabilizing agent for preparing gold nanoparticles. The supernatant was collected and stored at 4°C which was be used throughout all the experiments. The reaction was carried out at ph 7-8 at room temperature.

Green synthesis of Gold nanoparticles

To prepare gold nanoparticles, 25ml, 20ml, 15ml, 10ml and 5ml of seaweed extract was added to each 25ml of 1 milli molar (mM) aqueous Tetrachloroauric (III) acid trihydrate solution. 50ml of Tetrachloroauric (III) acid trihydrate solution without addition of seaweed extract served as the control set up. The container was tightly covered with aluminum foil to prevent photo reduction of gold ions, and incubated under dark conditions at room temperature. The solution was kept in magnetic stirring for constant stirring. The samples were stored for 24 hours to allow the complete reduction of metal ions. A color change of the solution change from brown to ruby red to purple color indicated the reduction of gold metal ion into gold nanoparticles. After the reaction reached saturation, 100ml of gold nanoparticles was centrifuged at 5,000rpm for 60 minutes. The obtained pellet was redispersed in distilled water to remove any uninterested biomass. The process of centrifugation and redispersion was carried out thrice to get a better separation of gold nanoparticles. The colloidal gold nanoparticles in the Erlenmeyer flask were covered with aluminum foil paper to prevent photodegradation at a temperature of 5-7° Celcius for further characterization.

Characterization of Gold nanoparticles

UV-Visible Spectral Analysis

The measurement of absorption spectra was done at Research and Laboratory Services (RLSC) of

SPAMAST-Malita. The bioreduction of the gold ions in the solution was monitored using UV-1800 Shimadzu UV Spectrophotometer by measuring the UV-vis spectra (400-700nm) of solutions. Quartz cuvette was used for the analysis. Distilled water was used for adjustment of the baselines. The deionized water was loaded and measure by the UV-vis spectrophotometer for baseline correction. 1ml of synthesized colloidal gold solution was measured after the color changed into ruby red or purple indicative of the synthesis of the Gold nanoparticles until complete reduction of gold ions was observed.

Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (SEM-EDS) Analysis

The characterization on the morphology of the gold nanoparticles was sent to Chemistry Laboratory of Ateneo De Davao University. The morphology of the nanoparticles and the composition of elements were confirmed by using the scanning electron microscopy with Energy dispersive analysis (SEM-EDS), Hitachi SU150. The electrons produced in SEM are produced by a FEG (Field Emission Gun) with a tungsten emitter which operates in a negative potential. The pseudo-image obtained is a result of the secondary and back scattered electrons generated upon interaction of the electrons with the sample.

The prepared gold nanoparticle pellet was mixed with 10ml deionized water and kept on sonicator to prevent aggregation of gold ions. The resultant solution was lyophilized. A drop of a very small amount of sample on a carbon coated copper grid and thin films of the gold nanoparticles was prepared. The thin films dried the sample and were analyzed.

FTIR Analysis

The aqueous suspension of gold nanoparticle was submitted to Analytical Science laboratory, Institute of chemistry, University of the Philippines, Diliman. The FTIR scan of gold nanoparticles sample was obtained using attenuated total reflectance (ATR) accessory to identify the functional groups present in the brown seaweed extract that probably act as the reducing agent of gold ions to nanoparticles. The final spectrum was processed using ATR correction.

Results and discussion

The development of Gold nanoparticles has potential application in various fields such as biosensors, catalysis, photonics, electronics, biomedicine and optics (Singaravelu *et al.*, 2007). The applications of gold nanoparticles are highly dependent on its chemical composition, shape, size and monodispersity. Moreover, the particles should be chemically stable without undergoing degradation, such as partial oxidation or undesired sintering. There are several physical and chemical methods employ for synthesizing metallic nanoparticles (Kruis, 1995).

However, the development of simple and eco-friendly and bio-compatible synthetic route would be highly desirable in the synthesis of metallic nanoparticles. In this regard, *S. polycystum* have shown to be an important biological component in the utilization for green synthesis of gold nanoparticles.

The extract brown seaweed *Sargassum polycystum* mediated the green synthesis of gold nanoparticles. The Fig. 1 illustrated the 5 known concentrations (25, 20, 15, 10, and 5ml) of *S. polycystum* extract added to 25ml Tetrachloroauric acid (III) trihydrate ($HAuCl_4$), a changed in color brown into ruby red to purple after 10, 21, 30, 72 and 152 minutes were observed. The color of ruby red was highly evident to 25, 20 and 15ml extracts while the purple color is highly conspicuous to the 10 and 5ml extracts of *S. polycystum*. The extract of brown seaweed *Sargassum polycystum* contain rich biomolecules of proteins, carbohydrates, lipids, fiber and ash (Perumal *et al.*, 2019) and phytochemicals namely tannins, flavonoids, terpenoids, cardiac glycosides, phlobatanins, steroids, phenols, and amino acids (Perumal *et al.*, 2019) that probably facilitated the bioreduction of Tetrachloroauric acid (III) trihydrate ($HAuCl_4$) into gold nanoparticles. Similar findings were reported in the bioreduction of gold nanoparticles using the extract of *Sargassum myriocystum* (Ismail *et al.*, 2018), *Sargassum crassifolium* (Ouano *et al.*, 2018), *Sargassum polycystum (*Sivaraj *et al.*, 2015) and *Sargassum wightii* (Oza *et al.*, 2012).

From Left to Right (A.) Synthesized gold nanoparticle in ruby red colour solution **(B.)** gold solution **(C.)***Sargassum polycystum* Extract

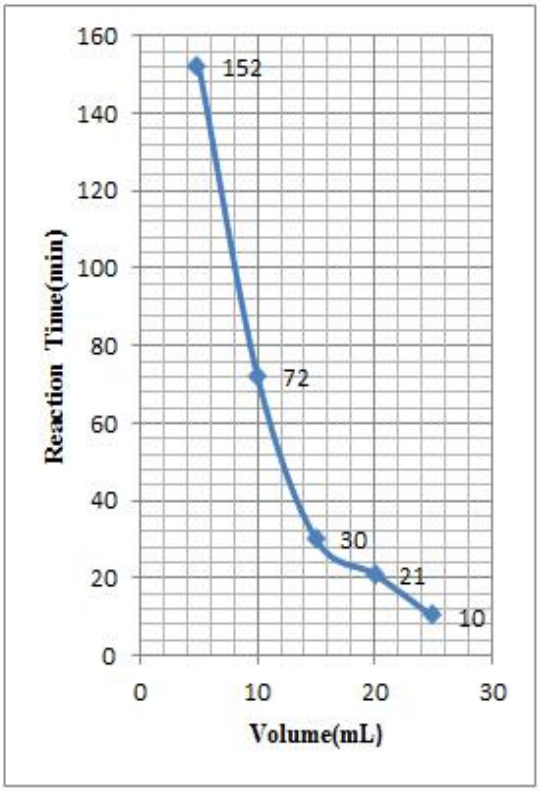

Fig. 1. Bioreduction reaction of Gold nanoparticles synthesized by plant extract of *Sargassum polycystum* (SP).

The bioreduction of gold nanoparticles from SP extract is concentration-dependent. Moreover, there is direct relationship between the volumes of *Sargassum polycystum* extracts towards the kinetics of gold nanoparticle synthesis. The higher the volumes of the seaweed extracts, the faster the reaction synthesis of gold nanoparticles. The extract of *Sargassum polycystum* contains various biomolecules that will act as reducing agents, stabilizing agents and capping agents. The same findings reported that the higher concentration of reducing agents probably increase the catalytic activity which facilitate faster reaction. Moreover, the higher concentration of *Sargassum polycystum* extract also increases the concentration of biomolecules that act as reducing agent, thus increasing the reaction rate which resulted rapid growth of gold nanoparticles (Das *et al.*, 2011), (Song *et al.*, 2009).

The successful formation of the gold nanoparticles was confirmed by the UV-vis spectroscopy. Spectral signatures of the five (Paul *et al.*, 2017) concentrations of *S. polycystum* mediated synthesized gold nanoparticles were detected in the different wavelengths shown in Fig. 2. The plant extract mediated synthesis of gold nanoparticles were confirmed by the following spectrum and absorbance properties, the known concentration (25ml) has spectral properties of 526nm, (20mL) has spectral properties of 530nm, whereas (15ml) has 534nm, meanwhile (10 and 5ml) has both 548nm.

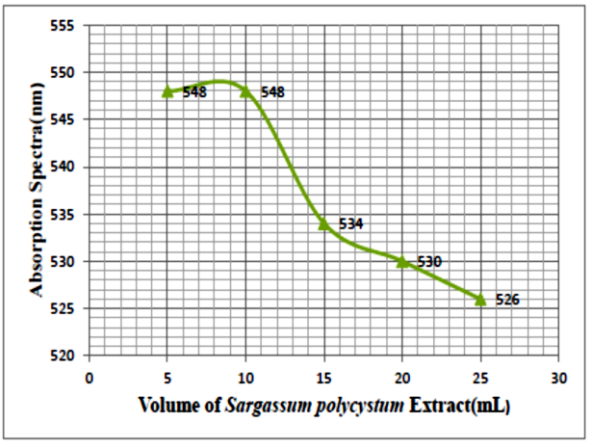

Fig. 2. Spectroscopic Analysis of Gold Nanoparticles synthesized by plant extract *Sargassum polycystum*.

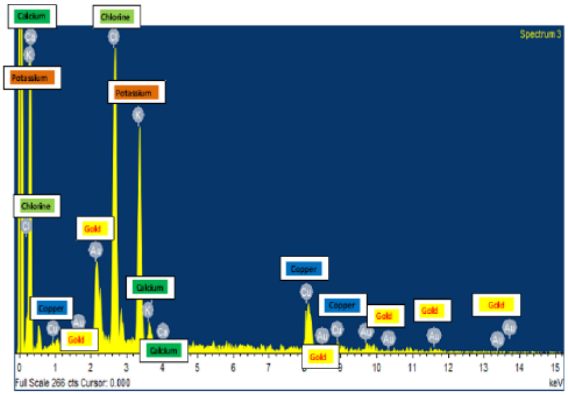

Fig. 3. EDS Spectra of gold nanaparticles synthesized by plant extract of *Sargassum polycystum*.

The broad peaks were observed at 526nm to 548nm that is the surface plasmon resonance of Gold nanoparticles. The visual color change is based on the principle of surface plasmon resonance (SPR). The color change occurs when the size of the particle increases, in the case of gold it is from ruby red to purple. The varying color changes were due to localized surface plasmon resonance (LSPR) that they exhibit, and it lies in the visible region of the electromagnetic spectrum, which means that the particular region of the wavelength in the visible region is absorbed while another gets reflected and emitted wavelengths will reflect its own color(Menon *et al.*, 2017).

The characteristic optical property exhibited of the metallic nanoparticles is due to the oscillations of the conduction band of electrons at the surface of the nanoparticles (Menon *et al.*, 2017). The optical properties of gold nanoparticles range from 500 to 600nm depending on its size.

The observed absorption spectra were in agreement on the reported synthesis on *Sargassum myriocystum* (Mohamed *et al.*, 2018) *Sargassum crassifolium* (Basilia *et al.*, 2018), *Azima tetacantha* (Abirami *et al.*, 2016) *Sargassum tenerrimum* (Rao *et al.*, 2016) Sargassum *wightii Greville* (Kumar *et al.,* 2007), *Turbinaria ornata* (Ashokkumar *et al.*, 2016) and *Turbinaria conoides* (Vijayan *et al.*, 2014), *Curcuma longa* (Prabhu, 2015), *Padina pavonica* (Geethu *et al.*, 2015) *Sargassum polycystum* (Ouano *et al.*, 2018), *Gracilaria corticata* (Sugandhi *et al.*, 2014), *Momordica charantia* (Pandey *et al.*, 2012). The elemental properties of the *S. polycystum* mediated synthesized gold nanoparticles were detected by the energy dispersive x-ray spectroscopy. The EDS peaks detected the presence of elemental gold (Azizah *et al.*, 2016) and other elemental residue. Each peaks indicating the presence of specific material in the sample. Au (Gold), Ca (Calcium), K (Potassium), Cl (Chlorine), and Cu (Copper) materials showed their characteristics excitation peaks in the sample region.

Notably, the excitation peaks of Au (Gold) are highly abundant in the brown seaweed extract. The morphology of the materials was characterized using the scanning electron microscopy (SEM) technique confirmed the synthesis of a gold nanoparticle (shown in fig. 4). The gold produced were a cluster of spherical gold nanoparticles with sizes ranging from 68.5 to 240nm. Accordingly, a material with a size of 1-100nm can be called as nanoparticles (Nadeem *et al.*, 2017).

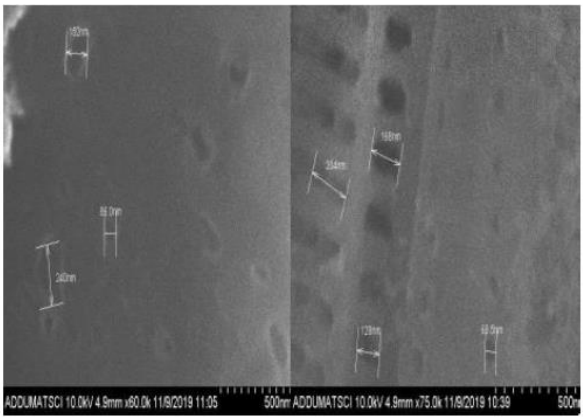

Fig. 4. Scanning electron microscope image of gold nanoparticle synthesized by plant extract of *Sargassum polycystum*.

The SEM image confirmed the development of gold nanostructures using the brown seaweed extract *S. polycystum*. The different sizes of the gold nanoparticles maybe attributed to the different compounds found in the *S. polycystum* that maybe responsible for the reduction and stabilization of gold nanoparticles (Basilia *et al.*, 2018).

Ultimately, the optical property of the synthesized material is highly indicative of a gold nanoparticle.

The optical properties of spherical gold nanoparticles are highly dependent on the nanoparticle diameters (Ogarev *et al.*, 2018) smaller nanoparticles primarily absorb light and have peaks near 520nm, while larger spheres exhibit increasing scattering peaks that broaden significantly and towards longer wavelengths known as red shifting (Basilia *et al.*, 2018).

The absorption spectrum of the synthesized gold nanoparticles is at 526 to 548nm at different volumes of *S. polycystum* extract. Moreover, the optical properties of gold nanoparticles change when particles aggregate and the conduction electrons near each particle delocalized and are shared among neighboring particles. When this occurs, the surface plasmon resonance shifts to lower energies, causing the absorption and scattering peaks to red shift to longer wavelengths (Basilia *et al.*, 2018). The un-aggregated gold nanoparticles exhibit to have a red color in the solution. If the particles aggregate, the solution will appear blue (Long *et al.*, 2009). The color of the colloidal gold shown in fig. 1 and the color change of the different volumes of the *S. polycystum* extract in appendix F.

The elemental morphology of the spherical gold nanoparticles was further confirmed by the EDS and usually carried out using the Tetrachloroauric acid (III) trihydrate ($HAuCl_4$).In the current work, $HAuCl_4$ is used as the precursor of gold nanoparticles (Usman *et al.*, 2019), (Alaqah *et al.*, 2016), (Koperuncholan, 2015), (Herizchi *et al.*, 2014).

The Fig. 5 shows the different FTIR spectral peaks of the aqueous extract of brown Seaweed. The first peak which is located in the 3,520-3,200 region shows an (O-H stretching); the second peak located in the 1,650-1,600 region indicates an (N-H bending), while the other peak is located in the 720-590 region indicative of a (O-H out of bending). The peaks denote the possible biomolecules present in the aqueous extract of *Sargassum polycystum,* which includes alcohols and primary amines (Coates, 2013).

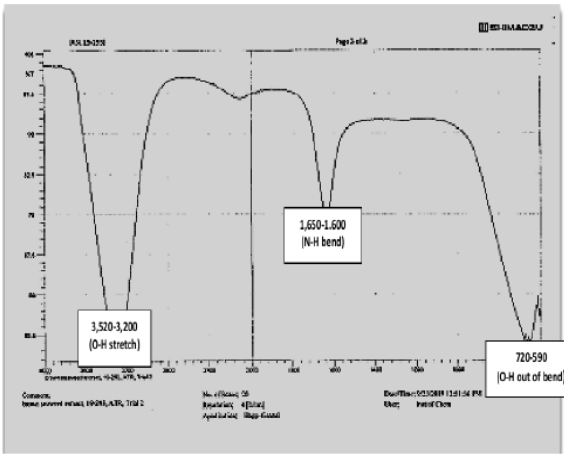

Fig. 5. FTIR Spectrum of gold nanoparticles synthesized by *Sargassum polycystum* extract.

The spectral measurements on aqueous extract of brown algae and the obtained gold nanoparticles was carried out to identify the possible biomolecules that are responsible for the reduction of Au (III) ions as well as those which acted as capping agents of the gold nanoparticles. The FTIR spectrum indicated the presence of different functional groups, which give rise to the various signatures in the IR region of electromagnetic spectrum. The Intense and broad absorption bands were detected at 3,520-3,200, 1,650-1,600 and 720-590cm^{-} . The peaks at 3,520-3,200 (O-H stretch) is a characteristic of a hydroxyl functional group in alcohol and phenolic compounds. The band at 1,650-1,600cm^{-} (N-H bend) can be assigned to the functional group of primary amines. Moreover, the band at 720-590cm^{-} (O-H out of bend) corresponds to Hydroxyl functional groups. A previous report reveals that the Hydroxyl group (O-H) has a strong ability to interact with nanoparticles (Sugandhi *et al.*, 2014), (Rao *et al.*, 2016), (Kanakalakshmi *et al.*, 2016).

This observation revealed that compounds having hydroxyl functional groups could possibly serve as the reduction agent as well as aid in the stabilization of gold nanoparticles (Ramakrishna *et al.*, 2015). Moreover, the presence of hydroxyl functional groups has been shown to be involved in the bioreduction of silver ions in the algae extract of *Sargassum polycystum* (Thangaraju *et al.*, 2012). Hydroxyl containing compounds are known to possess ability to interact with nanoparticles (Davis *et al.*, 2003).

Hydroxyl groups are very abundant in polysaccharides of the algal cell wall (Kuyucak *et al.*, 1989) and their involvement in the reduction process is highly probable. Algal pigments, such as fucoxanthin, a kind of carotenoids rich in hydroxyl groups, could also have participated in the reduction. Those pigments have reducing properties and are released in the solution by diffusion (Huang *et al.*, 2010). These soluble compounds could have acted as capping agents preventing the aggregation of nanoparticles in the solution. (Deepali *et al.*, 2015).

Moreover, hydroxyl group is found in the compounds of titerpinoids, which can be found in different species of brown alga (Ismail *et al.*, 2018). The presence of primary amines were also detected on the *S. polycystum* extract which is also in agreement on the previous work on the synthesis of gold nanoparticle using *Sargassum crassifolium* (Basilia *et al.*, 2018), and *Sargassum polycystum* for synthesis of silver (Sivaraj *et al.*, 2015) and gold nanoparticles (Deepali *et al.*, 2015).

The metal uptake capacity of a specific brown algae *Sargassum* species is highly documented to be an excellent candidate for nanotechnology studies. The following Sargassum species have notably higher content of alginic acid among Sargassum species namely the *Sargassum longifolium*, *Sargasum wightii*, *Sargassum tenerium, Sargassum fluitans*, *Sargassum oligocystum* ranging from 17% to 45% dry weight (Huang *et al.*, 2010), (Jerod *et al.*, 2015).

Previously, it was reported that brown algae *Sargassum natans* have shown efficient sequestration of Gold and Cobalt in the solutions outperforming the conventional method of ion-exchange (Huang *et al.*, 2010). Moreover, brown seaweed species of *Sargassum wightii*, *Sargassum ilicifolium*, *Sargassum linarifolium*, *Sargassum polycytum* metal uptake on Iron, Cadmium, Cobalt, Chromium, copper, lead, manganese, nickel and Zinc were documented (Jothinayagi *et al.*, 2009).

The bioreduction property of *S. polycystum* extract can be inferred to the excellent biosorption capacity of brown algae. Biosorption is a passive binding of heavy metals to the surface of the algal cell wall (Ozer *et al.*, 2005). The algal biomass is highly biosorptive due to its rich biochemical composition (Jeba *et al.,* 2016).

Macroalgae contain many poly-functional metal binding sites for both cationic and anionic metal complexes. Potential metal cation-binding sites of algal cell components namely carboxyl, amine, imidazole, phosphate, sulphate, sulfhydryl, hydroxyl and chemical functional groups contained in cell proteins and sugars (Podgorskii *et al.*, 2004), (Brinza *et al.*, 2007).

Brown algae received the most attention due to higher metal uptake capacity compared to red and green algae (Paknikar *et al.*, 2003). The alginic acid content of brown algae cell wall offers anionic carboxylate and sulfate ions at neutral ph. The brown algae biomass contained metal sequestering chemical like the acetamido groups of chitin, amino, sulfhydryl and carboxyl groups in amino acids and proteins, hydrohyls in polysaccharides (Romera *et al.*, 2006). Moreover, the cell walls of brown algae generally contain three components, cellulose which is the structural support; the alginic acid a polymer of mannuronic and guluronic acids and the corresponding salts of sodium, potassium, magnesium, calcium and sulphated polysaccharides. The carboxyl and sulphate groups are the predominant active groups (Gaur *et al.*, 2009).

The biosorption of the heavy metal ions on the cell surface occurs by ion exchange process. This was illustrated on brown marine alga, *Sargassum vulgaris,* and uptake on metals like Cadmium, Nickel and Lead. The participation of the main chemical groups on the algal cell wall such as carboxyl, amino, sulfhydryl and sulfonate is highly suggested (Gong *et al.*, 2005). The macromolecules of the algal cell wall provide abundant functional groups such as thio-ether, carboxyl, imidazole, hydroxyl, carbonyl, phosphate, and phenolic that can form coordination complexes with heavy metals (Hashim *et al.*, 2004).

Among the brown seaweeds, *Sargassum* species possesses relatively high metal binding capacity (Alluri *et al*., 2007). The mechanism involve in the biosorption is be based on cell metabolism. It may be metabolism dependent and non-metabolism dependent. Secondly, it can be classified based on the location of the sorbate species, it may be extracellular or intracellular accumulation (Ahluwalia *et al*., 2007).

The cell biomass of brown alga contains protein and polysacharrides which offer a lot of binding sites for heavy metals. The first step is the stoichiometric interaction between the cell components and the metal ions. Secondly, the accumulation of heavy metal on the binding sites (Herrero *et al*., 2006). The main uptake mechanisms are the Ion exchange between proteins and heavy metal ions at the binding sites or light metals and heavy metals (Li *et al*., 2006), (Han *et al*., 2006), adsorption by physical forces, electrostatic interaction (Gupta *et al*., 2006), chelation (Gupta *et al*., 2006) and micro-precipitation (Kaewsarn, 2002).

It is elucidated that biosorption on dead biomass involve a physical and chemical sorption phenomena plus transmembrane and accumulation of heavy metals in the cell. Biosorption happens when metal ion bud to the cell wall via an ion exchange and the metal ion is transported into the cellular interior (Ofer *et al*., 2004).

The ion exchange mechanism has been suggested as the dominant mechanism of absorption by algal biomass (Costa *et al*., 2003), (Aksu *et al*., 1992). Generally, the untreated cell biomass contains light metals such as potassium, sodium, calcium and magnesium which are bound to the acid functional group of the alga. In the biosorption process, the anions are exchange with heavy metals. The biosorption test for *Sargassum sp*. for Zinc uptake was reported. After the digestion, amount of light metals in stipe and blades were observed. It was concluded that alkaline and alkaline earth elements have been contributed on the biosorption of zinc (Silva *et al*., 2003).

The metal removal from solution can also take place via complex formation onto the cell surface as a result of the interaction between metal and active functional groups. The phenomenon involves the covalent coordination and link electrostatic forces. The study on the biosorption of copper by *Chlorella vulgaris,* the removal of copper involves the formation of coordination bonds of metals and amino and carboxyl groups of the cell wall polysaccharides (Mata *et al*., 2009).

Moreover, another plausible mechanism is the chelation which involves chelates. Chelates are complex formation of multi-dentate ligands. The binding sites can be inner sphere or outer spheres complex. The mechanism is largely covalent in character or chiefly electrostatic in nature. There are two possible scenarios involves in metal biosorption, the interacting ligands is immediately adjacent in the metal cation and the other one is when the ions of opposite charge are attracted and approach each other within a critical distance and effectively form an ion pair. In outer sphere complexes, the metal ion or the ligand are often separated by one or more water molecules. The live microalgae *Chlorella vulgaris* can bio-reduced the metal from its high valence state via complexing with polysaccharides then transported and accumulated inside the cells (Gupta *et al*., 2006).

Moreover, the biosynthesis, bioreduction, and biosorption is a connected in brown algae mechanism as reported on the species of *Fucus vesiculosus* and *Turbinaria conoides*. The biosorption and bioreduction of Au (III) to Au (0) using biomass of the brown alga *Fucus vesiculosus* were reported. Hydroxyl groups present in the algal polysaccharides were involved in the gold bioreduction. The metallic gold was detected as micro-precipitates on the biomass surface and in colloidal form as nanoparticles in the solution (Vijayaraghavan *et al*., 2011).

The biosorption mechanism was proposed to involve electrostatic interactions between gold anions and algal functional groups, when the *Turbinaria conoides* was exposed to gold solution, $AuCl_4$– anion binds to positively charged functional groups, such as amino groups (NH_2), on the algal surface.

The *T. conoides* reduced Au (III) to gold nanoparticles. The Hydroxyl groups present in the brown algal polysaccharides were involved in the bioreduction of Au (III) to Au (0) (Vijayaraghavan *et al.*, 2011).

The reduction of tetrachloroaurate with brown algae *S. polycystum* is an effective method for the synthesis of gold nanoparticles from dilute solutions: the method is cheap, easy performance at alkaline pH and room temperature using dead cell biomass and environmental friendly compared to other chemical methods that uses toxic chemicals. Additionally gold can be obtained as a high value added product based on its rich potential applications and their excellent properties. Gold nanoparticles can be an exceptional nanomaterial for detection of environmental pollutants.

Conclusion

Based on the result of the study, it is concluded that *Sargassum polycystum* (C. Agardh) contained the functionalities that act reducing agent, capping agent and stabilizing agent in the synthesis of gold nanoparticles. The functionalities identified were Hydroxyl groups and primary amines group. The Uv-Vis Spectrophotometer detected the wavelength property of the gold nanoparticles around the 526 to 548nm. Through SEM and EDS confirmed the gold nanoparticles with spherical morphology and particle sizes range of 68.5 to 240nm. Thus, the green synthesis of colloidal Gold nanoparticles using the Brown seaweed *Sargassum polycystum* (C. Agardh) was demonstrated. The synthesized gold nanoparticles can be further screened for potential biomedical or environmental applications.

Acknowledgement

We would like to thank the Department of Marine Biology of SPAMAST and Department of Biological Sciences of College of Science and Mathematics, Mindanao State University-Iligan Institute of Technology (MSU-IIT) for all the support in the conduct of the study. The corresponding author would like to express heartfelt gratitude to Dr. Mylene Uy, Dr. Rey Vequizo and Dr. Maria Luisa Orbita for sharing their expertise to improve the quality of the study.

Moreover, the corresponding author would like to thank the Department of Science and Technology-Accelerated Science and Technology Human Resource Development program (DOST-ASTHRDP) for the scholarship and research funding.

References

Abirami H, Nargis B, Muhammad I, Syed J, Premkumar K, Shilu M, Archunan G, Qadr I. 2016. Synthesis of Plant Mediated gold Nanoparticles using *Azima tetracantha* Lam. Leaves extract and Evaluation of their Antimicrobial Activities. Pharmacognosy Journal **Vol 8, Issue 4.**

Ahluwalia SS, Goyal D. 2007. Microbial and plant derived biomass for removal of heavy metals from wastewater-Review. Bioresource Technology **98 (2007)**, 2243-2257.

Aksu Z, Sag Y, Kutsal T. 1992. The biosorption of copper (II) by *Chlorella vulgaris* and *Zoogloea ramigera*. Environmental Technology **13,** 579-586. https://doi.org/10.1080/09593339209385186

Alaqah K, Saleh TA. 2016. Gold and Silver nanoparticles: Synthesis Methods, Characterization Routes and Applications towards Drugs. Journal of Environmental & Analytical Toxicology **Issue 4. Volume 6.** DOI: 10.4172/2161-0525.1000384

Alluri HK, Ronda SR, Settalluri VS, Singh J, Bondili Suryanarayana V, Venkateshwar P. 2007. Biosorption: An eco-friendly alternative for heavy metal Removal. African Journal of Biotechnology **6(25),** 2924-2931. https://doi.org/ 10.5897/AJB2007.000-2461

Arunkumar L, Sangappa KG, Shashidhar. 2019. Synthesis, characterization and antibacterial study of Ag–Au Bi-metallic nanocomposite by bioreduction using piper betle leaf extract. Heliyon 5. https://doi.org/10.1016/j.heliyon.2019.e02794

Ashokkumar T, Vijayaraghavan K. 2016.Brown seaweed-mediated biosynthesis of gold nanoparticles. Journal of Environment and Biotechnology Research **Vol. 2, No. 1,** Pages 45-50.

Azizah N, Hashim U, Arshad MD, Subash CB, Gopinath SH, Nadzirah MA, Farehanim MF, Fatin A K, Muaz AR, Ruslinda RM. 2016. Analysis study of single Gold nanoparticle system of Interdigitated Device Electrodes (IDES) by using energy-dispersive-Xray (EDX). ARPN Journal of Engineering and Applied Sciences **Volume 11, no. 14**

Balanquit BJ, Fuentes RG. 2015. Preliminary Phycochemical Screening and Antioxidant activity of some brown Algae *Sargassum* species from Lawaan, Eastern Samar, Journal of Nature Studies **14(1),** 12:21

Balawag B. 2016. Distribution Pattern of Macro-benthic Algae at the Sublittoral Zone in Alibungog, Barangay Tubalan, Malita Davao Occidental, Undergraduate Thesis, SPAMAST, Davao Occidental Philippines

Basilia BA, Ouano JJ, Alguno A, Que MC. 2018. Controlling the Absorption Spectra of Gold Nanoparticles Synthesized via Green Synthesis using brown Seaweed (*Sargassum crassifolium*).Key Engineering Material. ISSN: 1662-9795, **Vol 772,** pp 78-82. DOI: https://doi.org/ 10.4028/

Brinza L, Dring MJ, Gavrilescu. 2007. Marine microalgal and microalgal species as Biosorbents for heavy metals. Environmental Engineering and Management Journal **6,** 237-51

Calumpong HP, Menez EG. 1997. Field guide to the common mangroves, seagrasses and algae of the Philippines. Bookmark Incorporated

Coates J. 2013. Interpretation of Infrared Spectra. A Practical Approach, Coates Consulting, Newtown, USA. Page 6-14

Costa ACA, Tavares APM, França FPD. 2003. The release of light metals from brown seaweed (*Sargassum* sp.) during zinc biosorption in a continuous system. Electronic Journal of Biotechnology **4,** 125-129.
https://doi.org/10.4067/S0717-34582001000300007

Das RK, Gogoi N, Bora U. 2011. Green synthesis of gold nanoparticles using *Nyctanthes arbortristis* flower extract. Bioprocess and Biosystems Engineering, PubMed.

Davis TA, Volesky B, Mucci A. 2003. A Review of the biochemistry of heavy metal biosorption by brown algae. Water Research 37(4311-4330).

Deepali S, Suvardhan K, Krishna B. 2015. Biogenic synthesis of nanoparticles: A review. Arabian Journal of Chemistry. **Volume 12, Issue 8, December 2019,** Pages 3576-3600.

Dhas TS, Ganesh Kumar L, Stanley Abraham L, karthick V, Govindaraju K. 2012.*Sargassum myriocystum* mediated biosynthesis of gold nanoparticles. Spectrochimica Acta part A; Molecular Spectroscopy 99(97-101).

Gaur N, Dhankar R. 2009. Removal of Zn+2 ions from aqueous solution using *Anabaena variabilis*: Equilibrium and kinetic studies. International Journal of Environmental Research

Gavino C, Trono JR. 1997. Field guide and atlas of the seaweed resources of the Philippines. Published by Bookmark, Inc. ISBN 971-569-252-4

Geethu IR, Emilin R. 2015. Brown Algae mediated synthesis, characterization of gold nanoparticles using *Padina pavonica* and their antibacterial activity against human pathogens. International Journal of Pharm Tech Research (2015) **Vol.8, No.9,** pp 31-40.

Gong R, Ding YD, Liu H, Chen Q, Liu Z. 2005. Lead biosorption and desorption by intact and pretreated *Spirulina maxima* biomass. Chemosphere **58(1),** 125-30.
https://doi.org/10.1016/j.chemosphere. 2004.08.055

Gupta VK, Rastogi A, Saini VK, Jain N. 2006. Biosorption of copper (II) from aqueous solutions by *Spirogyra* species. Journal of Colloid and Interface Science **296,** 59-63.
https://doi.org/10.1016/ j.jcis. 2005.08.033

Han X, Wong YS, Wong MH, Tam NFY. 2006. Biosorption and bioreduction of Cr (VI) by a microalgal isolate *Chlorella miniata*. Journal of Hazardous Materials in press.
https://doi.org/ 10.1016 /j.jhazmat.2006.11.053

Hashim MA, Chu KH. 2004. Biosorption of cadmium by brown, green, and red seaweeds. Chemical Engineering Journal **97,** 249-255.

Herizchi R, Abbasi E, Milani M, Akbarzadeh A. 2014. Current methods for synthesis of gold Nanoparticles. Artificial Cells, Nanomedicine, and Biotechnology **44(2),** 596-602.

Herrero R, Cordero B, Lodeiro P, Rey-Castro C, Vicente MESD. 2006. Interactions of Cadmium (II) and protons with dead biomass of marine algae *Fucus* sp. Marine Chemistry **99,** 106-116.

Huang X, El-Sayed M. 2010. Gold nanoparticles: Optical Properties and implementations in cancer diagnosis and photothermal theraphy. Journal of Advanced Research **Volume 1, Issue 1,** Page 13-28. https://doi.org/10.1016/j.jare.2010.02.002

Ismail AM, Sheik J, Ali H, Parthasarathy M. 2018.Biosynthesis of Gold nanoparticles using *Sargassum myriocystum* evaluation of their Antibacterial Activity. International Journal of Pure and Applied Bioscience **6(1),** 1340-1350.
http:// dx.doi.org /10.18782/2320-7051.6291

Jeba SD. 2016. Biosorption of Selected Heavy Metals from Synthetic Metal Solutions and Battery Slag Leachate using *Sargassum myriocystum* Biomass. Shodhganga, a reservoir of Indian theses @ Inflibnet: http://hdl.handle.net/10603/127310

Jerod M, Vigneshwaran C, Velmurugan S. 2015. Macrophytes: A potent Bio-Sorbent for the Sequestration of heavy Metals. International Advanced Research Journal in Science, Engineering and technology **Vol. 2. Issue 10.**

Jothinayagi N, Anbazhagan C. 2009. Heavy metal monitoring of Rameswaram coast by some *Sargassum* species. American-Eurasian Journal of Scientific Research **Volume 4. Issue 2.** pp.73-80

Kaewsarn P. 2002. Biosorption of copper (II) from aqueous solutions by pre-treated biomass of marine algae *Padina* sp. Chemosphere **47,** 1081-1085.

Kanakalakshmi A, Janaki V, Shanthi K, Kannan SK. 2016.Biosynthesis of Cr (III) nanoparticles from electroplating wastewater using chromium resistant *Bacillus subtilis* and its cytotoxicity and antibacterial activity. Artificial Cells, Nanomedicine, and Biotechnology **45(7),** 1304-1309.

Kanimozhi A, Kala CP. 2015. Extracellular synthesis of Silver nanoparticles from a marine alga, *Sargassum polycystum* (C. Agardh) and their Biopotentials. World Journal of Pharmacy and Pharmaceutical Sciences **Volume 4, Issue 09,** 1388-1400

Khanna P, Kaur A, Goyal D. 2019 Algae-based metallic nanoparticles: Synthesis, characterization and applications. Journal of Microbiological Methods, 16392019) 105656.
https://doi:10.1016/ j.mimet.2019 105656

Kharissova OV, Dias HVR, Kharisov Bl, Perez VMJ. 2013. The greener synthesis of nanoparticles Trends in Biotechnology **31(4).**
DOI: 10.1016/ j.tibtech.2013.01.003

Koperuncholan M. 2015. Bioreduction of chloroauric acid (HAuCl4) for the synthesis of gold nanoparticles (GNPs): A special empathies of pharmacological activity. International Journal of Phytopharmacy **Vol. 5 (4),** pp.72-80.

Kruis FE, Fissan H, Peled A. 1997. Synthesis of nanoparticles in the gas phase for electronic, optical and magnetic applications- A Review. Journal of Aerosol Science **29(5),** 511-535.
DOI: 10.1016/S0021-8502(97)10032-5

Kuyucak N, Volesky K. 1989. Biosorbents for recovery of metals from Industrial solutions. Biotechnology Letter **Vol 10. No. 2.** 137-142

Li ZY, Guo SY, Li L. 2006. Study on the process, Thermodynamical isotherm and mechanism of Cr (III) uptake by *Spirulina platensis*. Journal of Food Engineering **75,** 129-136.
DOI: 10.1016/j.jfoodeng .2005.04.003

Long NN, Vu LV, Kiem CD, Doanh SC, Nguyet TC, Hang PT, Thien ND, Quynh LM. 2009. Synthesis and optical properties of colloidal gold nanoparticles. Journal of Physics: Conference Series 187 (2009) 012026. IOP publishing. 2012, **2(3),** 505-512. Pelagia Research Library
DOI: 10.1088 /1742-6596/187/1/012026,

Mata YN, Torres E, Blazquezml Ballester A, Gonzales F, Munoz JA. 2009. Gold (III) biosorption and bioreduction with the brown alga *Fucus vesiculosus*. Journal of hazardous Materials **166(612-618).**

Mittal R, Tavanandi HA, Mantri VA, Raghavarao K. 2017. Ultrasound assisted methods for enhanced extraction of Phycobiliproteins from marine macro-algae, *Gelidium pusillum* (Rhodophyta) Ultrasonic Sonochemistry **38,** 92-103.
DOI: 10.1016/j.ultsonch.2017.02.030

Mohamed I, Sheik H, Ali J, Parthasarathy M. 2018. Biosynthesis of Gold Nano Particles Using *Sargassum myriocystum* and Evaluation of their Antibacterial Activity. International Journal of Pure and Applied Biosciences **6(1),** 1340-1350. DOI: http://dx.doi.org/10.18782/2320-7051.6291

Nadeem M, Abbasi BH, Younas M, Ahmad W, Khan T. 2017. A Review of the green synthesis and antimicrobial applications of gold nanoparticles. Green Chemistry Letters and Reviews **Vol. 10. No. 4.** 216-227.

Ofer R, Yerachmiel A, Yannai S. 2004. Mechanism of biosorption of different heavy metals by brown marine macroalgae. Biotechnology and Bioengineering **87,** 451-458. DOI: 10.1002/bit.20136

Ogarev VA, Rudoi VM, Dement'eva OV. 2018. Gold Nanoparticles: Synthesis, Optical Properties, and Application. Inorganic Materials: Applied Research **volume 9,** pages134–140(2018).
DOI: 10.1134/ S2075113318010197

Ouano JJ, Alguno A, Que MC. 2018. Controlling the absorption of Gold nanoparticles via green synthesis using *Sargassum crassifolium* extract. Key Engineering Material. ISSN: 1662-9795 **Vol 765,** pp 44-48. DOI:10.4028/www.scientific.net/KEM.765.44

Oza G, Pandey S, Shah R, Sharon M. 2012. European Journal of Experimental Biology Menon S. Rajeshkumar S. Kumar SV.A review on biogenic synthesis of gold nanoparticles, characterization, and its applications. Resource-Efficient Technologies **Volume 3, Issue 4,** Pages 516-527.
https://doi.org /10.1016 /j.reffit.2017.08.002

Ozer A, Akkayaa G, Turabik M. 2005. Biosorption of Acid Red 274 (AR 274) on *Enteromorpha prolifera* in a batch system. Journal Hazard Materials **126,** 119-127.
DOI: 10.1016 /j.jhazmat. 2005.06.018

Paknikar KM, Pethkar AV, Puranik PR. 2003. Bioremediation of metalliferous Wastes and products using Inactivated Microbial Biomass. Indian Journal of Biotechnology **2,** 426-443.

Pandey S, Oza G, Ashmi GO, Mewada A, Madhuri S. 2012. Green Synthesis of Highly Stable Gold Nanoparticles using *Momordica charantia* as Nano fabricator. Archives of Applied Science Research **4 (2),** 1135-1141. Scholars Research Library.

Paul JP, Lawrence RAR. 2017. Phytochemical analysis of *Sargassum linearifolium* (Turner) C.Ag. (Brown seaweed) using UV-VIS, FTIR and HPLC. International Journal of Herbal Medicine **5(6),** 14-17.

Perumal B, Chitra R, Maruthupandian A, Viji M. 2019.Nutritional assessment and bioactive potential of *Sargassum polycystum* C. Agardh (Brown Seaweed). Indian Journal of Geo Marine Sciences **Vol. 48(04),** pp. 492-498.

Podgorskii VSTP, Kasatkina OG, Lozovaia. 2004. Yeasts- Biosorbents of heavy metals. Mikrobiol Z **66,** 91-103.

Prabhu SN. 2015. Green route synthesis of stable isotropic gold nanoparticles using leaf extract of Curcuma longa and their characterization. Advances in Applied Science Research **6(8),** 167-179.

Ramakrishna M, Dandamudi RB, Gengan RM, Chandra S, Nageswara, Rao G. 2015. Green synthesis of gold nanoparticles using marine algae and evaluation of their catalytic activity. Journal of Nanostructure Chemistry (6:1-3).

Rao GN, Ramakrishna M, Babu DR, Gengan RM, Chandra S. 2016. Green synthesis of gold nanoparticles using marine algae and evaluation of their catalytic activity. Journal of Nanostructure in Chemistry **volume 6,** pages 1-13.

Romera E, Gonzalez F, Ballester A, Blazquezml, Munoz JA. 2006. Biosorption with algae: a statistical review. Critical Review of Biotechnology **26,** 223-235.
DOI: 10.1080/073885 50600972153

Sarda A. 2018. Assesment Macrobenthic Algae in Tubalan Cove, Malita Davao Occidental. Undergraduate Thesis. SPAMAST. Malita Davao Occidental Philippines

Silva EA, Cossich ES, Tavares CG, Filho CL, Guirardello R. 2003.Biosorption of Binary Mixtures of Cr (III) and Cu (II) ions by S*argassum* sp. Brazilian Journal of Chemical Engineering **20,** 213-227.
https://doi.org/10.1590/S0104-6632200300

Singaravelu G, Arockiamary JS, Kumar VG, Govindaraju K. 2007. A novel extracellular synthesis of monodisperse gold nanoparticles using marine alga, *Sargassum wightii* Greville. Colloids S rface Biointerfaces. **57(1),** 97-101. Epub 2007 Feb 1. DOI: 10.1016/j.colsurfb.2007.01.010

Singaravelu G, Govindaraju K, Kumar G. 2007. A novel extracellular synthesis of monodisperse gold nanoparticles using marine alga, *Sargassum wightii* Greville. Article in Colloids and surfaces B: Biointerfaces. DOI:10.1016/j.colsurfb.2007.01.010

Sinurat E, Saepudin E, Peranginangin R, Hudiyono S. 2016, Characterization of fucoidan extracted from *Sargassum polycystum*. International Journal of Chemical, Environmental and Biological Sciences (IJCEBS) **Volume 4, Issue 1.**

Sivaraj R, Priya SVR, Rajiv P, Rajendran V. 2015. *Sargassum polycystum* C. Agardh Mediated Synthesis of Gold nanoparticles assessing its Characteristics and its Activity against Water Borne pathogens. Journal of Nanomedicine and Nanotechnology (6:3).

Song JY, Jang HK, Kim BS. 2009. Biological synthesis of gold nanoparticles using Magnolia kobus and Diopyros kaki leaf extracts. Process Biochemistry **Volume 44, Issue 10.** Pages 1133-1138.
https: //doi.org/10.1016/j.procbio.2009.06.005

Sugandhi S, Rani G. 2014. Biosynthesis and characterization of gold nanoparticles from *Gracilaria corticata*. Nanoscience and Nanotechnology **Volume 8 Issue 12**(475-481).

Tulo R. 2018. Assessment of Seaweed s in the Southern Part of Davao Occidental. Undergraduate Thesis. SPAMAST. Malita Davao Occidental Philippines

Usman AI, Aziz AA, Noqta OA. 2019. Application of green synthesis of Gold nanoparticles-Review. Journal Teknologi (Sciences & Engineering) **81,** 1 (171-182).
DOI: https://doi.org/10.11113/jt.v81.11409

Vijayan SR, Santhiyagu P, Singamuthu M, Ahila NK, Jayaraman R, Ethiraj K. 2014. Synthesis and Characterization of Silver and Gold Nanoparticles Using Aqueous Extract of Seaweed, *Turbinaria conoides*, and Their Antimicrofouling Activity. The Scientific World Journal. Volume 2014. Article ID 938272. https://doi.org/ 10.1155 /2014/

Vijayaraghavan K, Mahadevan A, Sathishkumar M, Pavagadhi S, Balasubramanian R. 2011 Biosynthesis of Au(0) from Au(III) via biosorption and bioreduction using brown marine alga *Turbinaria conoides*. Chemical Engineering Journal 167(223-227).