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Review Article

Biogenic Silver Nanoparticles/Clusters Synthesis Characterization by Scanning Electron Microscopy and 2D-3D Topographical Analysis by Mountain-®-Graphical Tool

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ABSTRACT

The microalgae were exposed to Silver nitrate (AgNO3) solution and screened for their suitability for the production of nano-silver (nano-Ag).The intracellular and extracellular enzyme are secreted by microalgae(Phormidium sp.).The microalgae extract contain various active biomolecules such as superoxide dismutase, oxidoreductases, NADH-dependent enzymes, active bio-molecules (lipid, exopolymers, peptides), some protein and pigment phycobiliproteins play a major role in the formation of nanocluster and structures in the nanometer range, confirmed by change the brown colour indicates silver nanoparticle (Ag-NPs) synthesis. Extracted biogenic silver nanoparticles and its characterization studies which is applied on the Scanning Microscopic field to analysis the 2D, 3D topographical structure. The Mountain -®-expert graphical tool play an important role in structural prediction, stereoscopic configuration analysis, stereoscopic reconstruction, lateral analysis, Contour lines, Micro valley studies, Polynomial map, Partial, analysis, extract profile study, area of hole,fractal analysis, continuous wavelets decomposition, PSD, Abbott curve, Rk parameters, morphological envelop studies, R and w calculation, distance measurement, default mini-doc and 2D roughness analysis.

Keywords: SEM; Mountain -®- graphical tool; 2D Roughness Analysis; Microalgae

INTRODUCTION

Microalgae are small, unicellular or multi-cellular organisms commonly found in different environment [1-4] such as fresh water, marine water, and surface of moist rocks, hypersaline lakes, coastal dunes, saline deserts, salt marshes, coastal hyper saline lagoons, saltern evaporation ponds, inland salt seas and springs. The Dead Sea or salt sea [5] has a salinity of 33.7 percent. This is approximately 10 times moresalty than seawater. Cyanobacteria are not commonly found in high salt concentration up to 25 percentage of sodium chloride saturation. There are exceptions in few organisms example is extremophilic microalgae [6-8] *Phormidium sp.* also found in the Dead Sea, The Marakkanam salt pans are the third largest producer of salt in Tamil Nadu.It is one such example for thalassohaline environment; it contains the salinity range of five to ten times saltier than seawater [150-300gm/l saltconcentration]. Life at high salt concentrations requires special adaptations of the cell's physiology [6].

The modern nanotechnologists are difficult to fazing the major issues in development of mono-disperse of nanoparticles. The biological method for synthesis of silver nanoparticles using *Phormidium sp.* is a reliable and one of the best methods. It's proposed by two step mechanisms; the first one is nucleation and second step is reduction mechanisms. The application of nanoparticles in cancer studies are classified in to diagnostic part and therapeutic practices. Nanoparticles are capable of adsorbing cytosolic protein on their surface that may regulate the intracellular protein and they can enhance the gene expression [9,10]. Nanoparticles are inducing the autophagy [cellular degradation].Autophagy [11] is the natural regulated mechanisms of the cell that remove unnecessary or dysfunctional components. Three kind of autophay are called as microautophagy, macroautophagy and chaperon mediated autophagy. Silver nanoparticles are capable of inducing autophagy through accumulation of autophagolysomes [12] in human ovarian cancer cells.

Aptamers [13,14] are biomarkers especially used in cancer

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diagnostics and treatment. The different kinds of apatamers are commercially available named as DNA, RNA [15] and peptide aptamers. The aptamers molecules are high sensitive and specific affinity to a group of promising recognition units that can exactly bind to target cells. They have been used in biosensing, drug delivery therapy, disease diagnosis [16]. Early diagnosis of cancer is the best method for disease prevention and treatment. Today's researchers are developing multiple types of biosensors used for the identification of cancer-related proteins, small molecules and cancer cells. However, most of them dependent the on antibody-antigen binding assays to capture and monitor target proteins, which often require a sandwich system to detect their targets. The direct and indirect Elisa methods are used to identify the target. This reaction is antigen antibody dependant mechanisms and the affinities of antibodies are varying from batch to batch, which create the difficulties in development of reliable sensors for diagnostics. To overcomes this limitations by aptamers. Freeman *et al.* introduced an aptasensor based technique for detection of VEGF [17]. In their work, FRET-based sensors, chemiluminescence aptasensors and CRET aptasensor are used to develop the VEGF-induced conformation change on the probe. By introducing exonuclease III in to the optical aptasensor system, an amplified fluorescent sensor was developed for the detection of VEGF with a detection limit of 5 pM. Cho *et al.* developed a single-step surface-induced fluorescent aptasensor targeting on VEGF 165 [18] and the biomarkers are most commonly used in cancer angiogenesis. Recently, Yin *et al.* introduced a label-free and turn-on aptasensor for detection of tumor cells and this detection method are based on the [19] fluorescent DNA-silver nanoclusters (AgNCs). The introductions of fluorescent nanocluster are facilitated to increase the brighter fluorescence and better photostability. Mean while, several fluorescent aptasensors are used to finds the tumor cell and tissue fluorescence imaging *in vitro* and *in vivo* [20]. Nanoclusters have been extensively studied by experimental, 3D computational [21,22] and theoretical calculation method [23,24] because of their great importance in medicine, biology, catalysis [25], optics and electronics.

In the field of nanomaterials, multi-metallic clusters and their compounds have increased interest for wider range of properties by mixing two or more chemical elements, which often exhibit enhanced catalytic reaction performance compared to those of the pure metals. Structures of nanoAu and nanoAg clusters [25-31] can be categorized into dihedral, icosahedra, face cubic centred and learytetrahedran and the strong nanocluster [21,22] bonding are tabulated (Table 1). Halopilic microalgae *phormidium sp.* was isolated from east coast region of India. It is producing the anti proliferative compound as flavone. The MCF-7 and A549 cells [32-34] are control the WIN/WNT canonical signalling pathway. The anti proliferative compound flavones are binds to the human tankyrase 2 protein or TNKS. The TNKS consist of 417 amino acid residues its secondary structure is made up of 4 alpha helices and 9 beta plated sheets were inter connected with small peptide molecules. D loop, E loop, F loop, G loopmini loop, telomeric loop and sam domain are active site of tankyrase protein and its play an important role in attachment of flavones with strong H_2 bond, covalent bond,electrostatic force and disulphide bond. These compounds are strongly binds with lipid-glycoprotein [WIN/WNT protein] to trigger the win canonical signal pathway. First mechanism of canonical win signalling is formation of dissociation complex. It is obtained by binding of five protein such as protein kinase B,Gsk3, auxin, APC and β-catenin. The β-catenin contain scaffolding region are allowing for binding of Gsk3.

Table 1: Stable confirmation of Au-Ag cluster assembly.

Strong Nanocluster	Number of atoms [Au-Ag]
Dihedral	n = 30, 32–40, 42, 44, 46–48, 52–54,
Icosachedral	Ag31 Au31
Face centered cubic	n = 43, 45 and 51
Leary tetrahedran	Ag 49Au49 and Ag50 Au50

The dissociation complex activates the β catenin protein. The activated β catenin protein are binds with transcription factor to turn on the gene signals to reduce the activity of APC and prevent the attachment between APC and Auxin. The cancer cells are able to producing different number of protein molecule that blocked the canonical WNT signalling pathway but the ligand flavones molecules are activates the canonical signalling pathway to regulate anti proliferative activity against to cancer cell.

Scanning Electron Microscope (SEM)

Scanning Electron Microscopy (SEM) [26,35], produces images of a sample by scanning the surface with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals that contain information about the sample's surface topography and composition. In a typical SEM, an electron beam is thermionically emitted from an electron gun fitted with a tungsten filament cathode. The beam passes through pairs of scanning coils or pairs of deflector plates in the electron column, typically in the final lens, which deflect the beam in the *x* and *y* axes so that it scans in a raster fashion over a rectangular area of the sample surface. The signals used by a scanning electron microscope to produce an image result from interactions of the electron beam with atoms at various depths within the sample. Magnification in a SEM can be controlled over a range of about 6 orders of magnitude from about 10 to 500,000 times. SEMs may have condenser and objective lenses, but their function is to focus the beam to a spot, and not to image the specimen. Using the signal of secondary electrons image resolution less than 0.5 nm is possible. For SEM, a specimen is normally required to be completely dry, since the living cells and tissues and whole, soft-bodied organisms require chemical fixation to preserve and stabilize their structure.

SEM with EDAX (Energy Dispersive X-ray Spectroscopy

EDAX [36] (or EDX) is an X-ray spectroscopic method for determining elemental compositions. EDX Analysis stands for Energy Dispersive X-ray analysis. The EDX analysis system works as an integrated feature of a scanning electron microscope (SEM). During EDX Analysis, the specimen is bombarded with an electron beam inside the scanning electron microscope. The bombarding electrons collide with the specimen atom's own electrons, knocking some of them off in the process. The atom of every element releases X-rays with unique amounts of energy during the transferring process. Thus, by measuring the amounts of energy present in the X-rays being released by a specimen during electron beam bombardment, the identity of the atom from which the X-ray was emitted can be established.

MATERIALS AND METHODS

Sample Collection

Silver nitrate ($AgNo_3$) Chemicals was purchased from Sigma-Aldrich

(India), The isolated Cyanobacterial species were cultured in BG11 medium under 3000 lux light intensity with static condition, for 12 h under illumination and 12 h under darkness, culture was shaken manually twice in a day. Microalgal cultures were harvested approximately after a production period of 21days and used for synthesis of biogenic silver nanoparticles. For further studies based on the morphological appearance and Taxonomic classification of the microalgal isolates were identified as *Phormidium sp.*

Synthesis of Silver Nanoparticles (Ag-NPs)

The culture of microalgal strain growing in BG11 medium were harvested by centrifugation and these pellets were washed with sterile water and sonicated in a Equitron Ultrasonic Cleaner for 15 min at maximum output and duty cycle. The extract was critically examined microscopically to ensure complete breakage of the cells; if the extract showed the presence of filaments/cells, steps of sonication were repeated. If needed, the extract was incubated at 2-4°C overnight to attain complete lysis of cells. The resulting cell-free extract was centrifuged at 10,000 ×g for 15 min and filtered through Whatman No. 1 filter paper to remove cell debris, if any for the synthesis of [27,28] Ag-CNPs, the cell extract was distributed equally (50 ml each) into two flasks. One flask, silver nitrate ($AgNO_3$) was added to attain a final concentration of 1 mM., other flask contained only cell extract and served as the positive control. Pure $AgNO_3$ solution (1 mM) was taken in a flask separately for the negative control. All the flasks were wrapped in aluminum foil to ensure complete darkness and incubated at 25°C in a shaker (100 rpm) for 72 h. During the incubation period change the brown colour indicates silver nanoparticle synthesis.

Scanning Electron Microscopy Technique:

The sample preparation of Sem by arc discharge method [37] Jeganand K rishnaraj]. This methods requires number of solution such as phosphate buffer A [$Na_2HPO_4 \cdot 12\ H_2O$ - 35.82 g/500ml or$Na_2HPO_4 \cdot 2H_2O$ -17.8 g/500 ml], phosphate buffer B [$NaH_2PO_4 \cdot 2\ H_2O$ - 15.6 g/500 ml or$NaH2PO4 \cdot H2O$ - 13.6 g/500 ml] and3% glutaraldehyde [50 ml 0.2 M phosphate solution + 12 ml 25% glutaraldehyde + 38 ml distilled water]. The procedure for preparation of biological materials contain (Table 2) different step such as fixation washing post fixation again washing dehydration crtical point drying and metal coating.

Moutain-®- expert graphical tool: Application keys

Convert image into surface: Converted in to luminance.

Convert the image in to a surface with a colour –Coded Z-scale; by specifying the size or the resolution of the Z-axis.

Lateral analysis: In studiable having a regular pattern detect a unit cell touantifythe scaling distortions and linearity distortions.

Single image reconstructiom: Estimate the tophography from a single image. [Oblique low –angle lighting: East-West].

Stereoscopic reconstruction 3D image: Reconstruct topography from stereoscopic pair of image.

Contour lines: 2D graph shoeing contour line of surface point lying at the height level; dividing the surface into regularly spaced at horizontal "slices".

Furrows: Analyze parameters concerning the micro valley network (Depth; Density...).

Remove form (polynomial degree): Remove a polynomial form; a sphere or a cylinder

Extract profile: Extract a cross section from the surface. The resulting extracted profile corresponds to the intersection of the surface and a vertical plane perpendicular to the surface.

Partial analysis: Detect and quantify particles; pores grains and other image features having boundaries. The study provides several feature detection method (threshold segmentation; ISO 25178-2 watershed segmentation; edge detection and circle detection) graphical representation and classification tools.

Area of hole: This method simply traces a line between the two summits from one side to another of the lowest point. This line is considered to be the top of hole.

Fractal analysis: Calculate the fractal dimension on the studiable, in order to describe itscomplexity in the form of a single number. The fractal dimension for a profile is a between1 [a line] and 2 [a line]. The fractal dimension for a surface is between 2 [a plane] and 3 [a volume].

Table 2: Process and Compounding.

Step	Process	Compounding [Table:2]
Step 1	Fixation	Immerse the sample in 3% Glutaraldehyde buffered with 0.1 M Phosphate buffer at room temperature or 0-4°C (2-4 hours, maximum 24-48hours).
Step 2	Washing	Rinse the tissue with 0.1 M Phosphate buffer pH=7.2 - (3 x 10min.).
Step 3	Post-Fixation	Immerse sample in 1-2% Osmium Tetroxide in 0.1 M Phosphate buffer, pH=7.2 (2-4 hours) at room temperature and in a light tight container. 1-2% Osmium Tetroxide Solution: (1%) - 0.25g OsO4 in 25 ml 0.1 M Phosphate buffer (12.5 ml 0.2M Phosphate solution + 12.5 ml distilled water) or 2% Osmium Tetroxide Solution: 0.25g OsO4 in 12.5 ml 0.1 M Phosphate buffer (6.25 ml 0.2M Phosphate solution + 6.25 ml distilled water).
Step 4	Washing	in 0.1 M Phosphate buffer pH=7.2 (3 times for 10 minutes).
Step 5	Dehydration	in a graded ethanol or acetone solutions in water – 30%, 50%, 70% (can store tissue in 70% ethanol), 80%, 90%, 96%, 100% for 5-15 minutes each); 2 times in 100% ethanol or acetone (15-30 minutes each).
Step 6	Dehydration	in a graded ethanol or acetone solutions in water – 30%, 50%, 70% (can store tissue in 70% ethanol), 80%, 90%, 96%, 100% for 5-15 minutes each); 2 times in 100% ethanol or acetone (15-30 minutes each).
Step 7	Critical Point Drying	10-20 minutes
Step 8	mounting	Mount on specimen stub with carbon/silver paste or graphite
Step 9	Metal coating	Coat with gold/palladium alloy
Step 10	Viewing sample	Store stubs in desiccators

Continuous wavelet decomposition: Overview of the complete spectral content of a profile; in a single space frequency representation; Study scale levels and spatial location where phenomena occur; using wavelets ;spatial location on the X-axis; scale of the wavelets on the Y-axis; Intensity of the phenomena on the Z-axis (using a color scale)

Averaged power spectrum density: Easy to read representation of the spectral content; with the X-axis graduated in wave length; and the Z-axis graduated in amplitudes.

Distance: Calculation of distance in nanopartcle by autogeneric tool

Abbott curve: Depth histogram allows you to observe the distribution of the depths of the data points. Abbott- Firestone curve otherwise called as Material ratio curve; cumulating function of the depth distribution. Often used to predict and evaluate wear characteristics.

Morphological envelop: Visualize the upper envelop [using a morphological closing filter along with the profile] and the lower envelop [using a morphological opening filter].

R and W: Display of the waviness and roughness motifs and of the upper envelop curve; as they are defined in the ISO 12085 standard. This standard used in the automotive industry aim at studying the functional aspect of the profile.

Rk parameters: Functional parameters Rk; Rvk; Rpk...are calculated from the Abbott curve.

2D roughness analysis: Macro including the following analysis steps; Analyse the depths distribution of the profile using an Abbott-Firestone study. On an extracted portion that you can define with the mouse. Display general information about the profile by inserting it's identity card.

Default minidoc: Macro including the following analysis steps; Display the waviness and roughness profile (use the button in the study's editing tool to switch between them) Abbott-Firestone curve; and a table containing amplitude and bearing ratio parameters.

RESULTS AND DISCUSSIONS

The microalgae samples were observed under microscope, photographed and morphologically identified micro algal such as *phormidium.sp.*

Phormidium usually forms flat, slimy mats of tangled filaments. The filaments are long, cylindrical, and may be curved or spiralled. Thin, firm, colorless sheaths adhere closely to out growths or appendages on *Phormidium* cells. The apical cells may have calyptra with more pointed, narrow or sperical than the other cells.

Analysis of silver nanoparticle in SEM

The obtained morphology revealed the fact that the synthesized silver nanoparticles are almost spherical silver nanoparticles obtained from SEM. The particles are monodisperse but seem agglomerated as this could be due to the fact that the presence of some important bio-organic compounds in the microalgal extract seems to act as a ligand which effectively stabilizes the formed silver nanoparticles. The 2D & 3D topographical blue print (Table 3) can achieved by using mountain -®- expert tool which covers the following art creation such as stereoscopic configuration analysis, stereoscopic reconstruction, lateral analysis, Contour lines, Micro valley studies, Polynomial map, Partial, analysis, extract profile study, area of hole, fractal analysis, continuous wavelets decomposition, PSD, Abbott curve, Rk parameters, morphological

Table 3: Analysis of silver nanoparticle in SEM.

SEM -Parameters	Biogenic silver nanoparticles-*Phormidium sp.*
Shape	spherical, crystalline
Size	60 nm to 344 nm
EDAX profile	Included the elemental composition of Ag [3kev]
3D luminance	Converted
Lateral analysis	Degree of nonlinearity and correction were calculated
Stereo confirmation	Occurred
3D-stereo reconstruction	Occurred
Contour lines	Formed.
Micro-valley net work	Maximum; minimum and mean depth values were calculated.
Extract profile studies	Done
Partial analysis	Number of particles; coverage; density and threshold detection were calculated.
Area of hole	Maximum depth; area of hole were calculated.
Fractral analysis	Find the fractural dimension.
Continuous wavelets decomposition	Fusion of high & low frequency images are carried out; respectively according to the root mean standard.
PSD	Dominant wave length; maximum amplitude were calculated.
Distance measurement	Calculations of distance were calculated.
Rk parameters	The values are calculated from abbott curve.
2D-roughness analysis	Kurtosis; skewness and Rdq values were calculated
Default mini-doc	Rz; Ra and Rq values and maximum; minimum length of Z-axis values were calculated.
ISO 4287-Roughness (S-L)	[Table:4]
F: [Workflow] Leveled (LS-line)	
S-filter (λs):	Gaussian 2.500 µm
L-filter (λc):	Gaussian, 0.8000 mm
Calculated on:	All λc (341)
Amplitude parameters	
Rp	1.949 GL
Rv	1.889 GL
Rz	3.838 GL
Rc	6.980 GL
Rt	25.28 GL
Ra	1.662 GL
Rq	1.929 GL
Rsk	-0.2075
Rku	1.732
Spacing parameters	
RSm	1.542 mm
Rdq	83.52°
Rmr	0.03864%
Rdc	3.150 GL
Information	

envelop studies, R and w calculation, distance measurement, default mini-docand 2D roughness analysis. The biogenic silver nanoparticles of 2D & 3D topographical blue print were calculated and report described in below. The scanning electron microscopy scanner are encounters the primary and secondary electron molecules from the nanoparticles. The captured signals are further converted in to electronic images by digital convertor. The SEM images modified in to 2D and 3D topographical blue print of interfaces by mountain-®-expert which covers the described the art creation are mentioned above. These arts creation are electronic digital coding in the multiple programming languages such as object oriented interfaces in Macinotosch or MS –DOS.

CONCLUSION

The salt tolerance microalgae *Phormidium sp.* was isolated from East – Coast region of India. The microalgae were able to produce more number of compounds such as fatty acid [Palmitic acid, Octadecanoic acid, Nonadecane-2,4-dione and Methoxyacetic acid, octadecyl ester]and anti-cancer compound [Flavones]. The physical and geochemical variations are enhancing the osmotic stress activity in the micro algal cell. The cells are responding to trigger the respiration enzyme. These enzymes play an important role in synthesis of biogenic silver nanoparticle. The SEM micrograph showed the synthesized biogenic silver nanoparicle are spherical in shape and well distributed with an average size 60 nm to 344 nm. EDAX gives qualitative and quantitative status of elements that may be involved in the formation of biogenic silver nanoparticles. The peak in silver region at 3keV and which is typical for the absorption of metallic silver nanocrystalline due to surface plasmon resonance. The 2D & 3D topographical blue print can achieved by using mountain -®- expert tool which covers the following art creation such as stereoscopic configuration analysis, stereoscopic reconstruction,lateral analysis, Contourlines, Micro valley studies, Polynomial map, Partial analysis, extract profile study, area of hole, fractal analysis, continuous wavelets decomposition, PSD, Rk parameters, morphological envelop studies, R and W calculation, distance measurement, default mini-doc and 2D roughness analysis . The biogenic silver nanoparticles of 2D & 3D topographical blue print interface measurement were calculated and report. The scanning electron microscopy scanner are encounters the primary and secondary electron cluster molecules from the nanoparticles. The captured signals are further converted in to electronic images by digital convertor. The SEM images are modified to 2D and 3D topographical blue print of interfaces by mountain-®-expert which covers the described the art creations are mentioned above. The electronic digital coding signals are written in the multiple programming languages such as Macinotosch [objective- c- interfaces] and MS –DOS. , we report a green chemistry approach which is an eco-friendly method for the synthesis of silver nanoparticles (Ag-NPs) by using *Phormidium sp.* that they are quite stable in solution and it is more important advantage than the other methods. Further, the synthesized biogenic nanoparticles would be used for biomedical application of cancer therapeutics, coating medicinal devices and dental material.

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REFERENCES

1. Siew-Moi P, Emienour MM, RangaRao A, NikMeriam NS, Phaik-Eem L. Checklist of microalgae collected from different habitats in peninsular Malaysia for selection of algal biofuel feed stocks. Malaysian J Sci. 2015:34:148-174.
2. Charpy L, Casareto BE, Langlade MJ, Suzuki Y. Cyanobacteria in Coral Reef Ecosystems: J Mar Biol. 2012;9.
3. Thajuddin N, Subramanian G. Cyanobacterial biodiversity and potential application in biotechnology. Curr Sci.2005; 89: 47-57.
4. Nedumaran T, Manokaran S. Cyanobacterial flora in some salt pans of Pudukkottai District, Tamilnadu, India. J Phytol. 2009;1:169-171.
5. Jordan KM, Tayel EH, Samer A, Khaled AN. Heavy Metals Distribution in the Dead Sea Black Mud. JJEES.2009; 2:50- 59
6. Aharon O. Microbial life at high salt concentrations: phylogenetic and metabolic diversity. Saline Syst. 2008: 4:2.
7. Prachi V, Paulina M, Avigad V, John B, Pramod W. Extremophilic micro-algae and their potential contribution in biotechnology. Bioresour Technol. 2015: 184:363–372.
8. Aharon O, Danny I, Muna H, Hanan M. Microalgae and cyanobacteria of the Dead Sea and its surrounding springs. Israel J Plant Sci. 2008:56:1-13.
9. Xi-Feng Z, Zhi-GL, Wei S, Sangiliyandi G. Silver Nanoparticles: Synthesis, Characterization, Properties, Applications, and Therapeutic Approaches. Int J mol sci. 2016;17:1534.
10. Edina CW, Andrew Z W. Nanoparticles and their applications in cell and molecular biology. Integr Biol. 2014; 6:9-26.
11. Rajeshwary G, Scott JP. Macroautophagy and Chaperone-Mediated Autophagy in HeartFailure: The Known and the Unknown. Oxid Med Cell Longev.2018.
12. Xi-Feng Z, Sangiliyandi G. Combination of salinomycin and silver nanoparticles enhances apoptosis and autophagy in human ovarian cancer cells: an effective anticancer therapy. Int J Nanomedicine.2016;11: 3655-3675.
13. Tsuneo I. Anticancer activities of silver ions in cancer and tumor cells and DNA damages by Ag+ - DNA base-pairs reactions. MOJ Tumor Research. 2017;1:8-16.
14. Mei L, Xiaocheng Y, Zhu C, Tong Y, Dandan Y. Aptamer selection and applications for breast cancer diagnostics and therapy. J Nanobiotechnology.2017;15:81.
15. Baby S, Pramod KY. Nucleic Acid Aptamers: Research Tools in Disease Diagnostics and Therapeutics. BioMed Res Int.2014:540451
16. Shin-ichiro H, Alberto H, Jiehua Z. Current Advances in Aptamers for Cancer Diagnosis and Therapy. Cancers.2018;10:1- 33.17.
17. Ronit F, Julia G, Amily Fang-ju J, Ja-an AH, Thomus H. OpticalAptasensors for the Analysis of the Vascular Endothelial Growth Factor (VEGF).Anal chem. 2012;84:6192-6198.
18. Hansang C, Erh-Chia Y, Raghu S, Ted A, Bearinger P. Lee.Single-Step Nanoplasmonic VEGF165 Aptasensor for Early Cancer Diagnosis. ACS nano.2012;6:7607-7614.
19. Jinjin Y, Xiaoxiao H, KeminW, Fengzhou X. Label-Free and Turn-on Aptamer Strategy for Cancer Cells Detection Based on a DNA-Silver Nanocluster Fluorescence upon Recognition-Induced Hybridization. Analy chem.2013:85:12011-12019.
20. Xu W, Jiao C, Min W, Julia X Z. Aptamers: Active Targeting Ligands

for Cancer Diagnosis and Therapy. Theranostics.2015;5: 322-344.

21. Geeta B, Negi DS. Antibacterial and photocatalytic activities of silver nanoparticles synthesized by litchi chinesis leaves extract. World J Pharm Res. 2017;6:755-766.

22. Andrea F, Sebastian C, Fernanda P, Lorena M. Computational Modeling of the Interaction of Silver Nanoparticles with the Lipid Layer of the Skin. J Nanotechnology.2018; pp9.

23. Jijun Z. Tight-binding molecular dynamic study of silver clusters. Int Centre Theo Phys. 1999:1-10.

24. Rene F. Theoretical study of the structure of silver clusters. J Chem phys. 2000;115:2165-2177.

25. Rongbin D, Sai T, Xia W, Yiqing X, Run C. Theoretical study of the structures of bimetallic Ag–Au and Cu–Au clusters up to 108 atoms. Royal society publishing; 2019: 6:190342.

26. Grobelny J, Tsai1 DH, Pradeep N, Cook RF, Zachariah MR. Mechanism of nanoparticle manipulation by scanning tunnelling microscopy. Nanotechnology. 2006;17: 5519–5524.

27. El-Rafie HM, El-Rafie MH, Zahran MK. Green synthesis of silver nanoparticles using polysaccharides extracted from marine macro algae. Carbohydr Polym.2013; 96:403–410.

28. Lavanya K, Santhi R, Annapurani S. Green Synthesis of Silver Nanoparticles from the Leaf Extract of *Volkameriainermis*. Int J Pharm Clin Res.2017;9: 610-616.

29. Saeed F, Bahram A, Abdelnassar M. Green Biosynthesis of Spherical Silver Nanoparticles by Using Date Palm (Phoenix Dactylifera) Fruit Extract and Study of Their Antibacterial and Catalytic Activities. Acta Chim Slov. 2017;64:129-143.

30. Thakkar NK, Mhatre S, Parikh RY. Biological synthesis of metallic nanoparticles. Nanomed Nanotechnol Biol Med.2009;6:257-262.

31. Yousefzadi M, Rahimi Z, Ghafouri V. The green synthesis, characterization and antimicrobial activities of silver nanoparticles synthesized from green alga *Enteromorphaflexuosa* (Wulfen) J. Agardh. Materials Letters. 2014:137: 1-4.

32. Juan EH, Kenya D, Romero C, Lizeth PA, Mauricio A, et al. Mexican Microalgae Biodiversity and State-Of-The-Art Extraction Strategies to Meet. Sustainable Circular Economy Challenges: High-Value Compounds and Their Applied Perspectives. Marine drugs. 2019:17:1-24

33. Sarfaraz A, Feroz K. 3D-QSAR, Docking, ADME/ Tox studies on Flavone analogs reveal anticancer activity through Tankyrase inhibition. Sci Rep.2019; 9:5414.

34. Alexander MB, Kevin CJ, Radu VS, Aarti S, Yashi A. EthanDmitrovskyand Sarah J Freemantle.Evidence for tankyrases as antineoplastic targets in lung cancer. BMC Cancer.2013;13:211.

35. Kalsoom A, Shahid AK, Sher BK, Abdullah M. Scanning Electron Microscopy: Principle and Applications in Nanomaterials Characterization S. K. Sharma (ed.), Handbook of Materials Characterization.2019;113;145.

36. Manuel S, Simone B, Harpreet KL, Rita B, Elena B. Energy Dispersive X-ray (EDX)microanalysis: A powerful tool in biomedical research and diagnosis. Eur J Histochem. 2018; 62:2841

37. Krishnaraj C, Jagan EG, Rajasekar S, Selvakumar P, Kalaichelvan PT. Synthesis of silver nanoparticles using Acalyphaindica leaf extracts and its antibacterial activity against water borne pathogens.Colloids Surf B Biointerfaces.2010;76:50-56.