

## **SYNTHESIS AND CHARACTERIZATION OF PALLADIUM NANOPARTICLES & ITS CONTRIBUTION IN PHOTOTHERMAL THERAPY OF CANCER**

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### **ABSTRACT**

*Nanotechnology is very useful in manipulating the matter that there is scope of improvement in technologies in order to secure the environment. Currently, the usage of nanotechnology can be seen in the commercial market in the form of nano devices and nano materials which are playing a revolutionary part in the implications of safety, health and advancement in technology. Metal nano particles is helpful in optics, electronics and medicines etc. Palladium metal is also used at large scale. The usage of palladium nanoparticles in green chemistry approach is increasing. During synthesis, palladium nanoparticles are environment friendly. The study is tried to explore the synthesis and characterization of palladium nanoparticles with borassus flabellifer L. leaf extract. To analyze the optical characteristics of palladium nanoparticles, ultra-violet spectroscopy was used. To measure the qualities of palladium nanoparticles, X-ray diffractometre was used. To record the image and size of palladium nanoparticles, TEM was used. Cyto-toxic property of palladium nanoparticles has been recorded with the help of micro-plate reader. The purpose of this paper is to synthesize the palladium nanoparticles with low cost and to improve the usage of palladium nanoparticles with enhanced optical properties.*

**KEYWORDS:** *Characterization, Commercial, Electronics, Optical, Synthesis, Toxic*

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### **Article History**

**Received: 10 Jul 2019 | Revised: 25 Jul 2019 | Accepted: 07 Aug 2019**

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### **INTRODUCTION**

The properties like physical and chemical properties of noble nanoparticles are observed to be excellent in nature. There are many applications like electronics, optics and bio-sensing etc. where the usage of nano particles is found to be huge. In most of the catalysis reactions, the metal Palladium is used on larger scale. The best examples are hydrogenation, oxidation and reduction of fuel cells. There are many methods like chemical reduction, laser method or seed-mediated which are used for the synthesis of nano particles of Palladium. These days, the trend of preparing the nano particles of Palladium with the help of green chemistry is increasing with the introduction of industrialization. Also, it is observed that the biological synthesis of the nano particles of Palladium is more cost effective and environment friendly as it doesn't yield any kind of toxic product during the process of synthesis. The organisms like prokaryotic organisms and complex eukaryotic are widely used for the purpose of yielding of nano particles biologically. These organisms have the feature to minimize the ions of metal to yield the nano particles of the metals and thus, they can be used as the natural nano-factories.

## **MATERIALS AND METHODS**

### **Materials**

For this purpose, ethidium bromide, acridine and Palladium chloride were used. The other materials which were used as the materials were penicillin-streptomycin, fetal bovine serum and fetal bovine serum.

### **Borassus Flabellifer L. Leaf Extract Preparation**

The collection of leaves of *B. Flabellifer* were collected. The leaves were allowed to wash with water then these washed leaves were dried at convenient temperature and after that these leaves were pulverized.

The leaf powder of quantity 5 gram was used with the mixture of 100ml of water and this mixture was then boiled for about 5 minutes. Then, Whatman papers were used to filter the extracts of leaves and these extracts were used for the fabrication of nano particles of Pd.

### **Synthesis of Palladium Nanoparticles**

For the synthesis of Palladium nano particles, the 5ml of extracts of leaves of *B. flabellifer* L. were mixed with the solution of Palladium Chloride at moderate temperature. Then, this mixture was stirred. The reduction of Palladium ions started converting into the nano particles of Palladium as the color of the solution changed from the light yellow to the dark brown. After that, the solution was allowed to purify with the help of centrifugation for about 10 minutes with a frequency of 14000 rpm. Distilled water was used to wash the sample and then the sample was dried at a temperature of 60 degree in an oven which was further used for the purpose of in-vitro studies.

### **Physicochemical Characterization**

The ultra-violet spectroscopy was used in order to analyze the optical characteristics of the nano particles of Palladium. Fourier transform infrared were used to analyze the nano particles of Palladium and the functional groups of *B. flabellifer* L. leaves were also evaluated later in the experiment.

Also, x-ray diffractometer was used to measure the qualities of the nano particles of Palladium. The obtained nano particles of the metal Palladium were then placed on the copper grids coated with carbon. 200 kV voltage was used to record an image and size of the synthesized nano particles of Palladium.

### **Cell Culture**

For the research purpose, the cancer cells of human breast were acquired and then these cells were supplemented with the antibiotics and bovine serum. At a temperature of 37, the flasks of cell culture were allowed to incubate in carbon dioxide. Then, these cells were used for the assessment of cyto-toxicity after getting the confluence of about 90% and then trypsinized.

### **Cytotoxicity of Palladium Nanoparticles**

MTT assay were used in order to assess the cyto-toxic properties of synthesized nano particles of Palladium. Then the work of the plantation of cancer cells of human breast was performed in 96-well plates with the help of MDA-MB. After one day, the cells were allowed to expose to the nano particles of Palladium and the concentrations of different levels were used for this purpose. The level of concentration was ranging from 0 to 67.5  $\mu\text{g/mL}$ . Then, these cells were exposed with laser for about 5 minutes. In each well, dye solution of MTT were added and incubated for about 6 hours. Then, the

process of dissolving of the formazan crystals was done for each and every well. Micro-plate reader was used in order to scan and record the absorbance.

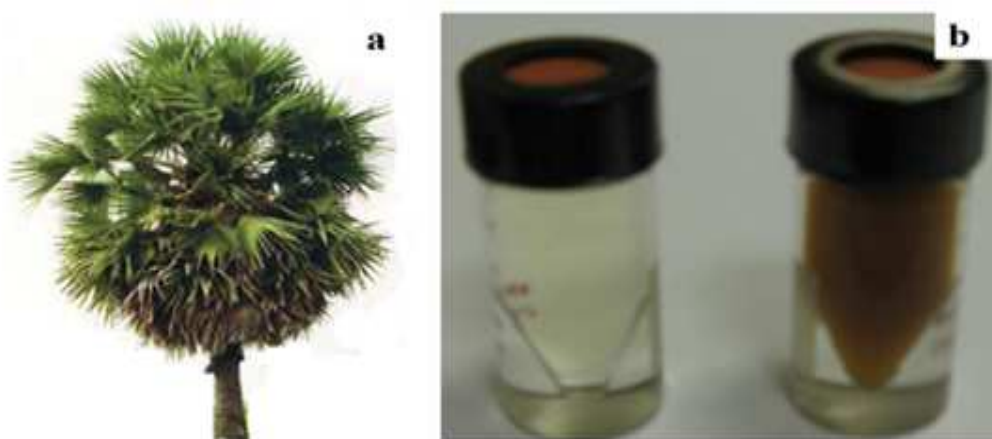
## RESULTS AND DISCUSSIONS

### Synthesis of Palladium Nanoparticles

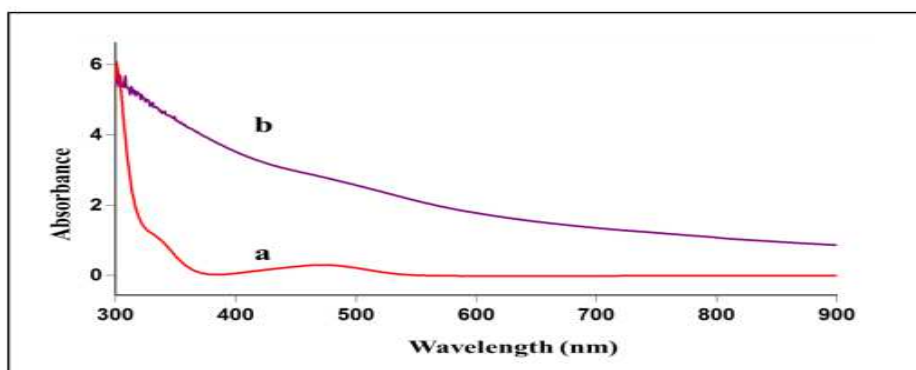
The approach of using the method of green chemistry for the preparation of nano materials has been successful in recent years. Many chemical ingredients such as tannis, saponins are found in the leaves of *B. flabellifer* L. The extracted leaves of *B. flabellifer* L. were then mixed with the ions of Palladium and it was observed that the color of the solution of Palladium Chloride has started to change from yellow to dark brown. This change in color indicated that there had been the reduction of ions of Palladium to the nano particles of Palladium.

### Physicochemical Characterization

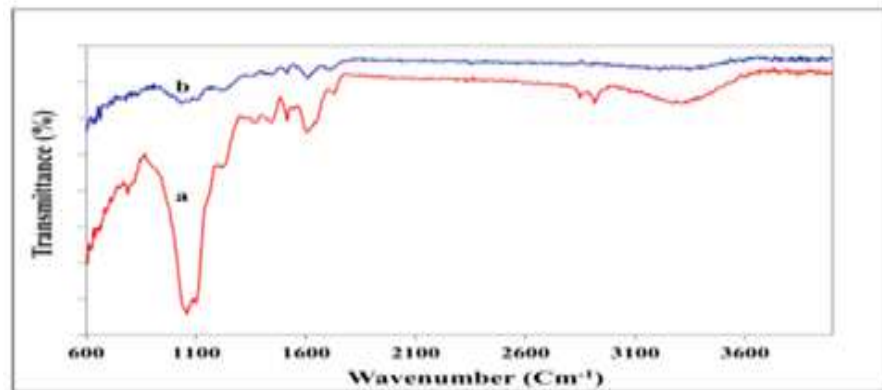
The below figure 2 suggests that the ultra-violet spectroscopy was used in order to monitor the process of the synthesis of the nano particles of Palladium. A quick absorption peak was observed in Palladium Chloride with respect to the ions of Palladium. It was observed that the peak intensity of Palladium ions decreased because of the vibration of surface plasmon after using the extracts of leaves. The sample of Palladium ions started to disperse due to the reduction of ions into Palladium nano particles.



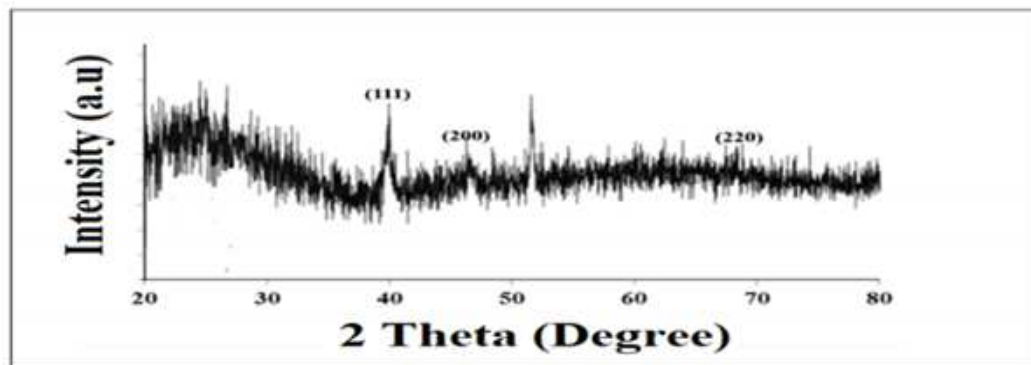
**Figure 1: A Digital Photograph of (a) Borassus Flabellifer Tree (b) PdCl<sub>2</sub> and Pd Nanoparticles Solution**



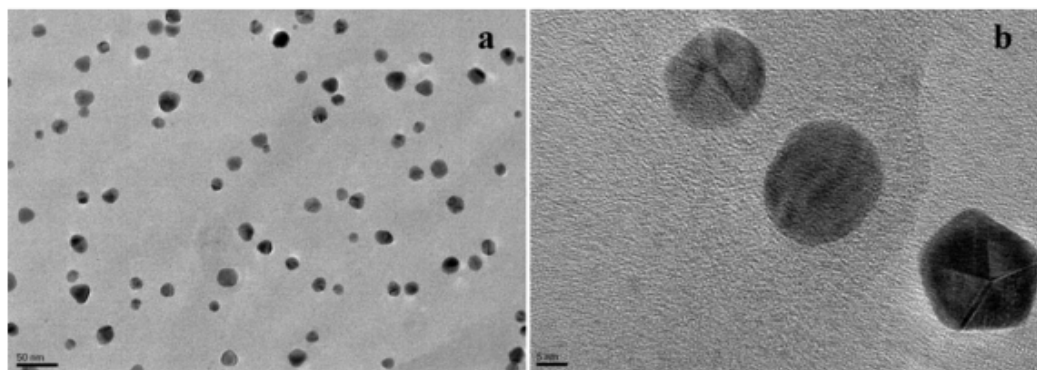
**Figure 2: UV-Vis Spectra (a) PdCl<sub>2</sub> and (b) Prepared Pd Nanoparticles**



**Figure 3: Fourier Transform Infrared Spectra of (a) Borassus Flabellifer Leaf Extract and (b) Synthesized Pd Nanoparticles**



**Figure 4: X-ray Diffraction Spectra of Synthesized Pd Nanoparticles using Borassus Flabellifer Leaf Extract**



**Figure 5: High-Resolution Transmission Electron Microscopic Images of Pd Nanoparticles at different Magnifications**

Hence, our study concluded that the extract of *B. flabellifer* L. leaves have the capability of perform the reduction of Palladium ions to the corresponding nano particles. Some past studies also suggested that the extracts of some other plants can also be used for the reduction of Palladium ions. In Figure 3, we showed the FT-IR results of extracts of *B. flabellifer* L. leaves and prepared Palladium nano particles. Here, the highest level of absorption peak was observed by the *B. flabellifer* L. leaf and this absorption peak was varied from 3100-3600 cm. These peaks of absorption were observed at

2920, 2851, 1735, 1607, 1515, 1446, 1376, 1362, 1221, 1101 and 1066  $\text{cm}^{-1}$ . C=O of esters, -NH<sub>2</sub> amino acids and OH bending of alcohols and phenols were observed at the absorption peak at 1735, 1607 and 1446  $\text{cm}^{-1}$ , respectively. On the other hand, C–O and C–H vibrations of esters and phenolic compounds were observed at the peak of absorption at 1376 and 1362  $\text{cm}^{-1}$ . Moreover, the absorption peak of C–O vibration and C–O and –OH was observed at 1221 and 1066  $\text{cm}^{-1}$ .

These results also indicated that there were many phyto-chemicals in the *Borassus flabellifer*. Here, the absorption peak points were observed at 1066, 1105, 1233, 1452, 1617 and 1643  $\text{cm}^{-1}$  in synthesized Pd NPs due to the *Borassus flabellifer* phytochemicals. The shape of synthesized nano structures of IR spectra was found to be similar. These results also showed the availability of phyto-chemicals in the extracts of *Borassus flabellifer* leaf Pd NPs. XRD spectroscopy was used to study the nature of the prepared Palladium nano particles. Figure 4 illustrates the XRD pattern of the synthesized Palladium nano particles. The reflections were observed at a temperature ranging from 40 degree to 68 degree Celsius. The committee was also satisfied with the XRD results of synthesized Palladium nano particles and suggested a structure of cubic crystalline which was faced to center.

HRTEM was used to study the structure of Palladium nanoparticles. Figure 5 represents the images of HRTEM of Palladium nanoparticles which were found spherical in shape having the diameter of 5–20nm. Then, these images were well-dispersed having no kind of integration level. The final result concluded that the extracts of *B. flabellifer* L. leaves can be used for the purpose of the synthesis of Palladium nano particles.

### Efficiency of Photothermal Therapy

It is observed that PTT is capable of converting the light energy into heat energy with the help of light sensitized materials. These materials were allowed to absorb the radiation in order to reduce the damage of healthy cells.

The properties of nano particles of Palladium related to the photo-thermal conversion were analyzed with the help of laser of length 808 nm. For the purpose of analysis of photo-thermal conversion, the dispersions of Palladium nano particles were synthesized. After the process of irradiation, the temperature of nanoparticles of Palladium was varied from 24–57 degree Celsius in order to observe the vapour droplets of water on the wall cells. It was concluded that the nano particles of Palladium are efficient enough to convert light energy into the heat energy and can be used as an excellent agent for cancer therapy. The MTT assay was used to study the impact of nano particles of Palladium on the viability of cells. Different level of concentrations were used to analyze the nano particles of Palladium for about one day.

It was observed that nano particles of Palladium were able to reduce the viability of MDAMB-231 cells. After the treatment of nano particles of Palladium, it was found that the viability tend to be 94%. The nano particles of Palladium thus can be used to reduce the viability level of cells. Some studies suggested that the nano particles of some metals can be used to induce the death of cells in a significant manner. These nano particles can also be used for the purpose of cancer therapy as some kind of efficiency is found for photo-thermal conversion which is very feasible for the task of cancer therapy agent. These cells were further used with these particles. After the task of incubation, these cells were allowed to expose to the laser of 808 nm. Fluorescence microscopy was used to observe the morphology of cells. The disintegration in the irradiated cells was found with the help of PTT. The viability of cancer cells was also checked during this process. Hence, it can be said that the Palladium nano particles can be used as PTT agent for the cancer therapy.

The synthesis of the nano particles of Palladium was done and the shape of these particles was found to be spherical and the diameter was observed to be 2–10nm. Then the study of the efficiency of the conversion of the nano particles of Palladium was performed. The 70% of cell death was induced with the help of the nano particles of Palladium

due to laser irradiation. During this study, it was concluded that the Palladium nano particles can be used for the purpose of cancer therapy. For the cancer therapy, these Palladium nanoparticles can be used with the association of doxorubicin (Dox)-loaded mesoporous silica NPs. The heat generated from the endocytosis as a result of the conversion of infrared light is found to be synergistic in nature.

The loadings of these sheets were performed in order to improve their characteristics so that these can be used for the photo-thermal cancer therapy in an efficient way. The nano-plates comprising of Silver and Platinum can also be used for the purpose of cancer therapy. The nano-devices based on Palladium can also be used for the cancer therapy initiated by the photo-thermal and photo-dynamic ways. A photo-sensitizer was also used for cancer therapy in the presence of photons. This resulted into the synthesis of groups of oxygen which were observed to be reactive in nature which caused some kind of damage to the cells.

A mice beared by tumor was given an intra-tumoral injection with the irradiation of Chlorine e6 and Palladium nano-sheets. This resulted in destroying the tumor. It was also examine that when irradiation was used in alone then it was not capable of destroying the tumor. The impact of Palladium and Platinum nanoparticles with the usage of laser irradiation was observed on the photo-sensitizer loaded with the silica and carboxyl phthalocyanine. A single beam from laser was used for the initiation of photo-therapy and it is a convenient way to use for the medical applications. Two laser beams were allowed to focus on a particular point on a patient. The Palladium nano-structures show the better photo-stability and these require lower density of the laser power as compared to other metals to be used as therapy agent. Many features of Palladium nano-particles like efficiency of converting photo-thermal, size and bio-compatibility make these Palladium nano-particles to be used as an excellent agent for the purpose of photo thermal cancer therapy. The cases of cancer are increasingly these days with the introduction of environment related problems and problems related to the safety of food. According to a survey, 12 million people are diagnosed with cancer worldwide and about 7.6 million people died of cancer. This data is increasing year by year. The safety of people is significantly threatened by the cancer disease. Among all type of cancer, the lung cancer, liver cancer are the most vital as many deaths are caused due to these type of cancer where the internal factors also associated. The therapies like chemo-therapy and radiation therapy are used in order to cure the cancer patients. These types of therapies are efficient for the cancer diagnosis found in earlier stage. Radio-therapy and chemo-therapy are widely used for the treatment of advanced cancer where the level of tumor is found to be critical and serious. As the side-effects of these treatments, a huge amount of damage is observed in the human body as radiation is used in this kind of cancer therapy.

In case of chemo-therapy, it is seen that some anti-cancer drugs are not found to be water soluble and have lower stability. In these cases, the drugs are not easier to digest and affected by macrophages before it can reach to the required tumor tissue. Hence, most of the doses are found to be less effective. Also, the drug carriers are found to be less targeted and many drugs penetrate into the tissues and organs. The silver nano-particles are found to have better physio-chemical features as compared to other metal nano-particles. Hence, they can be used for the medical applications. Also, the impact of silver nano-particles on the inhibitory bacteria like is higher. Also, the optical properties and absorption peaks of silver nano particles are observed to be localized and their quality factor tends to visible and the infrared bands of silver is visible to the ultraviolet light as compared to the nano-particles of other metals. The silver metal is also cost-effective as compared to other metals and is widely used as nano-silver. Silver nano-particles also show the tendency of the effect of Raman scattering enhanced by surface which is generally an abnormal surface which is used for the optical applications. Hence, the silver nano-particles are used as sensor.

The absorption ability of nano-silver is also observed to be higher under infra-red light and these nano particles also have the tendency to show their photo-thermal effects with the help of resonance effects of plasma over the surface of particles. The resonance among the free electrons is observed when light is emitted onto the silver nano-particles. These electrons absorb the light energy and the formation of electron gas takes place. The dot-matrix of silver nano-particles receive the energy transferred by the electron gas. Then, this energy is allowed to transfer in the atmosphere which certainly causes the increase in the external temperature. As a result, the nano-dendrites are formed which can be further used as sensitizers in photo-thermal cancer therapy. The cancer cells are killed with the help of photo-thermal activities by monitoring and initiating the preparation conditions controlled by the morphology and size of nano-particles.

Also, the efficiency of the conversion of photo-thermal tends to improve by the mixture of Palladium with the silver nano-particles which are good agent for the absorption of infra-red light which make it easier to use these particles for medical applications. The synthesis based on aerosol was also used for photo-thermal cancer therapy by simple reaction and raw materials with the progressive evaluation. The biocompatibility and the efficiency of photo-thermal effect can be improved with the help of silver nano particles. The bio-logical toxicity of platinum is found to be excellent as in case of silver. The bio-compatibility of Platinum is also observed to be better to be use for the cancer therapy. Oxygen reduction reactions are also carried out with the help of the nano-particles of Platinum where enzymes are also used to search the groups of reactive oxygen in cancer cells. Green method was used by Wang et al. in order to prepare the nano-particles of Platinum and these particles were tended to exhibit lower cyto-toxicity as compared to other metals and hence, it can be used for cancer therapy. At the tumor points, these particles have the efficiency of reaching to a temperature of 50 degree where the photo-thermal potential is supposed to be excellent in case of buffered saline solution. Tumor heterogeneity is used for cancer treatment. The stages of cancer are found to be different from one another and the cancer cells are not identical to each other in the profiles of genetic mutations where the mixture of stem-cells is malignant. These features certainly help the researcher that they can use these nanoparticles for future research work done in the field of medical from photo-thermal cancer therapy to the therapeutic efficiency and non-specificity. The heterogeneity of cancer is also initiated by some inorganic nanoparticles which can be further used for the cancer therapy under some particular surrounding of tumor where cancer cells are needed to be killed.

For therapeutic methods, the tumor cells are needed to have some interaction with the stromal cells so that higher permeability can be achieved which results in the vasculature of micro-environments for the tumor cells.

The effect of retention is also very critical at this stage as this effect can be used to target the cancer cells by using the nano-particles where these particles are appropriate because of their efficiency level for anti-cancer agents and can be conjugated in the accumulation of tumor cells. Hence, these nano-particles play an important role for cancer therapy. The role of EPR effect is very important as it is required for the targeted delivery of pressure of fluid in the tumor. The therapeutic efficiency can be improved with the help of anti-cancer therapy. For this purpose, nano conjugates can also be used.

## CONCLUSIONS

Initially, the nano structures of Palladium were not supposed to be a good agent for Cancer therapy. Hence, the formation of Palladium nano sheets was performed to make it available for the cancer therapy. These nano sheets are found to be size-dependent and tunable in nature so that it can be comfortably used for the purpose of cancer therapy.

These nano sheets having the edge of about 41nm were capable of killing the cancer cells with the help of a laser after 5 minutes. Also, a level of biocompatibility was shown when there was no irradiation. The photo stability of Palladium nano structures is observed to be higher than that of silver. The properties of Palladium nano-sheets like the efficiency of converting the photo-thermal can be improved with the help of coating of surface and functionalization. It is also observed that the efficiency of porous Pd nano particles to be the agent of photo-thermal is higher than that of silver. These nano-sheets can also be used for the reduction of coated palladium nano-sheets having the diameter less than 10 nm. An extended circulation of blood is observed in case of Palladium nano-sheets when there is no availability of irradiation. On the other hand, the ablation in tumor was observed when irradiation was used with the help of laser emitting infrared light. Renal clearance was also found in case of Palladium nano-sheets because of their size limitation. Due to filtration limit of renal, these Palladium nanosheets can be used for photo-thermal cancer therapy. Also, the therapies like photo-thermal chemodynamic and photo-dynamic can be performed with the help of nano-devices which can be also used as an agent for the photo-thermal cancer therapy.

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