

**Bio fabrication of gold and graphene nano particles –
Anticancer and Solar cell application**

S.SANTHIYA

15PCH011

**A Dissertation submitted to
Avinashilingam Institute for Home Science and
Higher Education for Women
(University Estd.u/s 3 of UGC Act 1956)
Coimbatore- 43.**

**In partial fulfilment of the requirement for the
Master's degree in Chemistry**

April 2017

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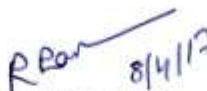
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T.H.17
Signature of the
Supervisor


8/4/17
Signature of the
Head of department

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LIST OF ABBREVIATIONS AND ACRONYMS

AFM	Atomic Force Microscopy
TEM	Transmission Electron Microscopy
PWD	The Public Works Department
mg	Milli gram
Kg	Kilo gram
nm	Nano meter
m	Meter
GNPs	Gold nano particles
S EM	Scanning Electron Microscopy
DC	Direct Current
DTA	Differential Thermal Analysis
DLS	Dynamic Light Scattering
MALDI-TOF	Time - Of- Flight Mass Spectrometry
NMR	Nuclear Magnetic Resonance
NTA	Nanoparticles Tracking Analysis
AgNPs	silver nanoparticles
FTIR	Fourier Transform Infra Red (spectroscopy)
RGO	Reduced Graphene Oxide
µg	Microgram
mL	Milli litre
DSSC	Dye-Sensitized Solar Cell
UV	Ultra Violet
XRD	X-Ray Diffraction

INTRODUCTION

1. INTRODUCTION

Plants are essential to balance the nature and people's lives (<http://www.infoplease.com/encyclopedia/science/plant-importance-plants.html>).

Research on plants enriches our intellectual life and adds to our knowledge about other life processes. The results of research on plant systems also can teach us how to approach problems in agriculture, health, and the environment. They are important in regulating climate, chemical and biologic conditions of the soil and water. Photosynthetic plants are the source of the fossil fuels and they provide the most readily harvested source of renewable energy for tomorrow (**National Center for Biotechnology Information, 2016**).

Due to the advantages of plants in terms of availability, ecofriendliness, non-toxic nature, plants have been chosen in the present study.

Currently the world's environment is greatly affected by pollution. Pollution is the major problem for living organisms and others. Water is the main source for living organisms for their survival. Water is polluted by various means such as industries, factories, human beings, animals, thermal etc. ***Eichhornia crassipes*** or water hyacinth is a free-floating perennial aquatic plant seen plentifully in the tropical water bodies. Each plant can produce thousands of seeds each year, and these seeds can remain viable for more than 28 years. ***Eichhornia crassipes*** are vigorous growers known to double their population in two weeks (<http://www.flowersofindia.net>). This invasive weed poses multiple hazards ranging from ecological and economical to social. It tends to endanger biodiversity, causes eutrophication, shelters pests, clogs fresh waterways, affects agriculture and aquaculture, hampers shipping and recreational activities (**Patel, 2012**).

Indian Government has been spending lots of money for removing ***Eichhornia crassipes*** and it is thrown as waste. It is a worldwide problem and it causes pollution to water bodies throughout the world. In February 2016 (**The Hindu, Feb 24, 2016 Page No-2**) our government has approved Rs.30 lakhs for cleaning the ***Eichhornia crassipes*** in Singanalloor lake at Coimbatore city. The government has spent 20.90 lakhs for the same purpose in February 2015 (**The Hindu, Feb 2, 2016**). The Public Works Department (PWD) had sent proposal for removing the weeds or hyacinth in the river Cauvery (**The Hindu, Dec 9, 2016**).

The Boat house in Singanalloor Lake in Coimbatore district, Tamilnadu, India was started with the aim of creating a tourist spot with aesthetic appearance. For the last few years this boat house is not functioning due to the algal bloom of ***Eichhornia crassipes*** which has stripped off the lake from its aesthetic appearance. ***Eichhornia crassipes***

decays in a short period deteriorating the water body, affecting the growth of flora and fauna. It also acts a store house of micro organisms.



Figure 1. Paper clipping- The Hindu, Feb 24, 2016 Page No-2

Eichhornia crassipes is an excellent source of biogas because of its high content of hydrocarbons (Times of India, Feb 11, 2013), (Shanab *et al.*, 2017). *E. crassipes* is an effective and inexpensive biomaterial for dye removal from aqueous dye solutions and industrial effluents (Wanyonyi *et al.*, 2013). The bleached ***Eichhornia crassipes*** is an efficient adsorbent for cationic dyes (Zawahry *et al.*, 2016), used in the removal of organic and inorganic pollutants from waste water (Shanab *et al.*, 2017) and determining soil type (Wang *et al.*, 2017). Water Hyacinth is used for making handicrafts at Lake Alaotra, Madagascar (Rakotoarisoa *et al.*, 2016) and for the removal of nitrate (NO₃⁻) (Jaya *et al.*, 2015). The methanol extract shows mild anticancer activity (Lenora *et al.*, 2015). The antimicrobial activity of different extracts (Kayathri *et al.*, 2015) and anti-inflammatory activity on formaldehyde induced pawo edema in Male Swiss Albino mice is reported (Jayanthi *et al.*, 2013). The reducing power of the aqueous extract and fractions – ethanol, aqueous, methanol and aqueous- of water hyacinth evaluated for their reducing power capability, crude root extracts (final concentrations 0.25–2.5%) showed 100% efficiency. The methanolic leaf extract (50%) at different doses (200 mg/kg body weight to 500 mg/kg body weight) showed good response against B16F10 *in vivo* melanoma tumour bearing hybrid mice models (from Swiss Albino female and C57BL male). Some fractions exhibited selective anticancer activity against liver cancer cell lines, while other fractions exhibited high anticancer activity against hormone dependent tumour types (cervix and breast cancers) (Tulika *et al.*, 2015).

Considering the aforesaid environmental problems initiated by water hyacinth it is necessary to remove it from the water bodies to save our environment. Also

there are many pharmaceutical applications of the plant. Hence this plant has been chosen for the study.

Nanotechnology provides a new platform for innovation in all fields of research, particularly within the context of meeting the challenges involved in the move toward greener and more eco-friendly processes. Recent developments in nanoscience have already played an important role in reducing the formation and emission of certain pollutants. Furthermore, other developments in the field of nanotechnology have led to an upsurge in the commercial uptake of several types of manufactured nanoparticles. These nanoparticles and nanomaterials have been applied in various fields, such as in electronics, sensors, and even in the biomedical space, due to the immense range of useful and diverse properties that are intrinsic to certain nanoparticles **(Khenfouch *et al.*, 2016)**.

The earliest application of nanoscale materials occurred in systems where nanoscale powders could be used in their free form, without consolidation or blending. For example, nanoscale titanium dioxide and zinc oxide powders are now commonly used by cosmetics manufacturers for facial base creams and sunscreen lotions. Nanoscale iron oxide powder is now being used as a base material for rouge and lipstick. Paints with reflective properties are also being manufactured using nanoscale titanium dioxide particles. In biomedical areas, structures called liposomes have been synthesized for improved delivery of therapeutic agents. Liposomes are lipid spheres about 100 nanometers in diameter they have been used to encapsulate anticancer drugs for the treatment of AIDS-related Kaposi's sarcoma. Several companies are using magnetic nanoparticles in the analyses of blood, urine, and other body fluids to speed up separation and improve selectivity. Other companies have developed derivatized fluorescent nanospheres and nanoparticles that form the basis for new detection technologies. These reagent nanoparticles are used in new devices and systems for infectious and genetic disease analysis and for drug discovery **(<https://www.nap.edu/read/10395/chapter/3#10>)**.

Even today various disease like diabetes, cancer, Parkinson's disease, Alzheimer's disease, cardiovascular diseases and multiple sclerosis as well as different kinds of serious inflammatory or infectious diseases (e.g. HIV) constitute a high number of serious and complex illnesses which are posing a major problem for the mankind. Nano-medicine is an application of nanotechnology which works in the field of health and medicine. Nano-medicine makes use of nano materials, and nano electronic biosensors. In the future, nano medicine will be beneficial to molecular nanotechnology. The medical area of nano science application has many projected benefits and is potentially valuable for all human races **(Nikalje *et al.*, 2015)**.

Hence in the present work it is aimed to synthesize nano particles and explore their captivating applications in technological and pharmacological areas.





The chemical and physical methods are widely used in the synthesis of nano particles. Bio-mediated synthesis methods have been suggested as alternative ways to produce noble metal nanoparticles. These simple techniques have low costs and can be conducted in eco-friendly ways indeed; various approaches to the biosynthesis of gold nanoparticles (GNPs) have been demonstrated. These include the use of micro-organisms, fungi, or plants. In the bio-mediated techniques, the organisms can be used to achieve active uptake of gold ions from a solution, reduce them to neutral gold atoms, and form gold nanoparticles. Plant extracts are used in a simple, low-cost, and bio-mediated approach for the production of nanostructures with low cytotoxicity levels (***Klekotko et al., 2016***).









A metallic nanoparticle has widespread use in biology, pharmacy and medicine and hence biosynthetic methods are being considered to prepare these nanoparticles. The use of plant extracts has gained great importance due to the fact that most of the plants are generally inexpensive, available, and nontoxic. Moreover, plant extracts are rich in different types of reducing and capping agents. Since gold nanoparticles are considered more biocompatible than other metallic nanoparticles, research studies performed on green synthesis of gold nanoparticles using plant extracts and different applications of these nanoparticles have been reviewed and discussed (***Noruzi et al., 2015***).

In the present study the synthesis of gold nano particles has been attempted by an eco-friendly method. A comprehensive search of literature survey for green synthesis of gold nano particles using various plant extract unveiled sparse work with Eichhornia crassipes plant extracts. Accordingly this plant was chosen for the present work.

Research is always re-searching to end up in the newer findings. Hence various methods are used to prepare gold nano particles such as, at room temperature, high temperature, sonication, pH variation method, solar method and optimization of the method by concentration variation of gold chloride and that of plant extract.

There are several techniques available to confirm the formation of gold nano particles. Few common techniques include:

-  Transmission Electron Microscopy
-  Scanning Electron Microscopy
-  Atomic Force Microscopy(AFM)
-  Dynamic Light Scattering(DLS)






-  X-Ray Photoelectron Spectroscopy
-  Powder X-Ray Diffraction(XRD)
-  Fourier Transform Infrared Spectroscopy(FTIR)
-  Matrix –Assisted Laser Desorption / Ionization
-  Time - Of- Flight Mass Spectrometry(MALDI-TOF)
-  Dual polarization Interferometer
-  Nuclear Magnetic Resonance(NMR)
-  Nanoparticles Tracking Analysis(NTA)for tracking of the Brownian motion

Selected aforesaid techniques have been employed in the present study to confirm the formation of nanoparticles.

It is the foremost task of a research to explore its applications once a novel material or procedure is found. Literature survey revealed gold nanoparticles to possess anticancer potential. Carbon nanoparticles and graphene are used in drug delivery applications. Hence it is attempted to explore the anticancer potential of the synthesized nanoparticles and graphene.

‘Cancer’ - the word still conjures up deep fears of a silent killer that creeps up on us without warning. Cancer, evoking such desperation has become a metaphor for grief and pain, a scourge straining our intellectual and emotional resources. The numbers are such that we come across a patient, a family member or a friend frequently. There are over 20 million people living with cancer in the world today. The term cancer is used generically for more than 100 different diseases including malignant tumors of different sites (such as breast, cervix, pros-tate, stomach, colon/rectum, lung, mouth, and leukemia, sarcoma of bone, Hodgkin disease, and non-Hodgkin lymphoma). Common to all forms of the disease is the failure of the mechanisms that regulate normal cell growth, proliferation and cell death. Ultimately, there is a progression of the resulting tumor from mild to severe abnormality, with invasion of neighboring tissues and, eventually, spread to other areas of the body.

Cancer is a leading cause of death group worldwide and accounted for 7.4 million deaths (around 13% of all deaths) in 2004. The main types of cancer including

-  Lung (1.3 million deaths/year)
-  Stomach (803,000 deaths)
-  Colorectal (639,000 deaths)
-  Liver (610,000 deaths)
-  Breast (519,000 deaths).

More than 70% of all cancer deaths occur in low- and middle-income countries. Deaths from cancer worldwide are projected to continue rising, with an estimated 11.5 million deaths in 2030 (**World Health Organisation, 2009**). Nanotechnology provides tremendous opportunities for multimodal, site-specific drug delivery to these disease sites and gold nanoparticles further offer a particularly unique set of physical, chemical and photonic properties with which to do so (**Dreaden et al., 2012**).

Green synthesis of silver nanoparticles (AgNPs) using the aqueous extract of dried **jujube fruit** and coating onto the graphene oxide (GO + AgNPs) showed potential cytotoxic activity against human cervical cancer cell line (HeLa) (**Sreekanth et al., 2016**). The cytotoxic effects against MKN-28 (Adenocarcinoma), Hep3B (Hepatocellular carcinoma), and MG-63 (Osteosarcoma) cells were evaluated using tetrazolium-based assay (**Pati et al., 2016**). *Mappia foetida* leaf extract mediated gold nanoparticles conjugated with activated folic acid and doxorubicin complex are found to be toxic for human cancer cells viz., MDA-MB-231, HeLa, SiHa and Hep-G2 (**Yallappa et al., 2015**). *Andrographis paniculata* Leaf extract mediated gold nanoparticles have significant effect on HeLa (human cervical cancer) and MCF-7 (human breast cancer) cell lines (**Babu et al., 2012**).

This study reveals the scope for Water hyacinth mediated nanoparticles in several applications and its applications are aimed at extending to the field of pharmacology. A composite form with gold nanoparticles and graphene would be prepared and its anti cancer efficiency tested against cancer cell lines.

Nowadays graphene is universally known as a promising material. Hence, the development of eco-friendly synthesis methods for this material is of great importance. Around the world, research institutions are trying to develop ways to revolutionize the production of graphene sheets of the highest quality. One of the most cost effective ways is possible by the reduction of graphene oxide into RGO (Reduced graphene oxide).

Xanthomonas oryzae pv. oryzae (Xoo) is a representative phytopathogenic bacterium causing bacterial infections in rice. The antibacterial activity of graphene suspended in different dispersants against Xoo was performed. Graphene oxide (GO) exhibits superior bactericidal effect even at extremely low dose in water (250 µg/mL), killing almost 94.48 % cells, in comparison to common bactericide bismethiazol with only 13.3 % mortality. The high efficiency in inactivating the bacteria on account of considerable changes in the cell membranes caused by the extremely sharp edges of graphene oxide and generation of reactive oxygen species, which may be the fatal factor for bacterial inactivation (**Chen et al., 2013**).

The multiple graphene-based composite materials developed for antimicrobial applications is provided, with an analysis of the different chemical fictionalization routes

used to modify graphene and graphene oxide with biocidal compounds. Graphene-based nonmaterials are used in the development of novel antimicrobial surfaces and coatings. Finally, promising avenues for material development are identified and critical questions surrounding graphene-based nonmaterials are discussed, providing a guide for future development and application of antimicrobial graphene-based materials (**Soroush et al., 2016**). The potential applications of graphene-based materials are increasingly recognized for their special physico-chemical and biological properties. In particular, graphene and graphene oxide as the foundation of nanocomposites have garnered much interest among researchers in many fields (**Liu et al., 2016**).

Reducing graphene oxide is achieved in a number of ways. Though they are all methods based on chemical, thermal or electrochemical, the most commonly employed methods to chemically reduce graphene oxide to graphene use hydrazine or its derivatives as the reducing agent. However, they are highly hazardous and explosive (**Lee et al., 2014**). Due to this environmental safe eco-friendly methods are required.

The synthesis and application of reduced graphene oxide occupies the highest position in the field of research prompted to make an attempt to prepare it using *Eichhornia crassipes* and exploring its pharmacological application.

Energy problem will be the top critical problems that humans may face in the coming 50 years, which was pointed out by Professor Smalley of Rice University in the USA (**Hamakawa, 2002**). China intends to spend more than \$360 billion through 2020 on renewable power sources like solar and wind (**Asia Pacific, 2017**). Solar energy is the most readily available source of energy. It does not belong to anybody and is also the most important of the non-conventional sources of energy because it is non-polluting and, therefore, helps in lessening the greenhouse effect. India receives solar energy equivalent to over 5000 trillion kWh/year, which is far more than the total energy consumption of the country (<http://edugreen.teri.res.in/explore/renew/solar.htm>). A solar cell is an electronic device which directly converts sunlight into electricity. Light shining on the solar cell produces both a current and a voltage to generate electric power. Many attempts have been made by several researchers to prepare solar cells of higher efficiency. Solar cell can be of different types such as Micro morph Cells (Tandem-Cell Using a-Si/ μ c-Si), Monocrystalline Solar Cell (Mono-Si), Multijunction Solar Cell (MJ), Nanocrystal Solar Cell, Perovskite Solar Cell ,Photo electrochemical Cell (PEC),Polymer Solar Cell ,Polycrystalline Solar Cell (Multi-Si),Quantum Dot Solar Cell, Thin Film Solar Cell (TFSC) (**Bagher et al., 2015**).

The Dye-Sensitized Solar Cell (DSSC) provides a technically and economically credible alternative concept to present day p–n junction photovoltaic devices. In contrast to

the conventional systems where the semiconductor assume both the task of light absorption and charge carrier transport the two functions are separated here. Light is absorbed by a sensitizer, which is anchored to the surface of a wide band semiconductor. Charge separation takes place at the interface via photo-induced electron injection from the dye into the conduction band of the solid. Carriers are transported in the conduction band of the semiconductor to the charge collector. The use of sensitizers having a broad absorption band in conjunction with oxide films of nanocrystalline morphology permits to harvest a large fraction of sunlight. Nearly quantitative conversion of incident photon into electric current is achieved over a large spectral range extending from the UV to the near IR region. Overall solar (standard AM 1.5) to current conversion efficiencies (IPCE) over 10% have been reached. There are good prospects to produce these cells at lower cost than conventional devices (**Grätzel, 2003**). Commercial applications, which were held up due to chemical stability problems, are forecast in the European Union Photovoltaic Roadmap to significantly contribute to renewable electricity generation by 2020 (**Bagher et al., 2015**).

Natural dye as well as organic dyes can be used in the DSSCs. Natural dyes obtained from plant parts, fruits and vegetables are used as dyes of higher efficiencies (⁽¹⁾)of power conversion of DSSCs devices has achieved above 10% (**Zeng et al.,2010,Qin et al.,2008:Horiuchi et al.,2004:Koumura et al.,2006:Snaith et al.,2009**). The use of synthetic dyes as sensitizer in DSSC provide better efficiency and high durability, but they suffer from several limitations such as higher cost, tendency to undergo degradation, and usage of toxic materials. These limitations have opened up for alternate sensitizers that are bio compatible natural sensitizers. Natural sensitizers contain plant pigments such as anthocyanin, carotenoid, flavonoids, and chlorophyll that are responsible for chemical reactions such as absorption of light as well as injection of charges to the conduction band of TiO₂ by the sensitizer. Therefore, dyes containing these pigments can easily be extracted from natural products like fruits, flowers, leaves, seeds, barks etc and can be employed as sensitizer for DSSC (**Shalini et al., 2015**).

As energy crisis is a major problem today, solar cell is expected to overcome this problem especially DSSC. Considering the applications of gold nanoparticles and reduced graphene oxide prepared using Eichhornia crassipes, it is intended to use these nanopartilces as the coating materials in solar cells and study their efficiency.

1.1 Objectives of the study

The important objectives of the work done on” **Bio fabrication of gold and graphene nano particles – Anticancer and Solar cell application** “are given below:

- To analyze the previous work done on solar cell fabrication, gold nano particles and reduced graphene oxide from various important e-resources.
- To prepare Graphene oxide by the use of oxidizing method.
- To prepare reduced graphene oxide with the use of *Eichhornia crassipes* plant extract.
- To determine the surface morphology of the RGO by recording the scanning electron microscope (SEM).
- To synthesize the gold nano particles with the use of *Eichhornia crassipes* plant extract.
- To prepare the gold nano particles using Oven heating method with *Eichhornia crassipes*.
- To confirm the formation of gold nano particles using UV spectroscopy.
- To analyze the pharmacological application of the graphene and gold nano particles synthesized via *Eichhornia crassipes*.
- To determine the anti- proliferative efficiency of the synthesized nano particles by MTT assay.
- To fabricate a low cost dye sensitive solar cell using reduced graphene oxide, gold nano particles and its composite and aqueous extract of water hyacinth respectively by using various light sources.
- To measure the current density and voltage flow through the prepare solar cell using various prepared compounds.

REVIEW OF LITERATURE

1. REVIEW OF LITERATURE

A review of literature aids to carry out a research work in a systematic manner. A detailed review on synthesis of gold nano particles, its pharmacological application and solar cell fabrication has been carried out. The consolidated review work is given in the following pages.

2.1 Taxonomic Hierarchy of *Eichhornia crassipes*

The species was discovered in 1823 by the German naturalist C. von Martius (**Télliez et al., 2008**). According to the **Integrated Taxonomic Information System (ITIS)** the Taxonomic Hierarchy of *Eichhornia crassipes* is given below,

Kingdom	Plantae – plantes, Planta, Vegetal, plants
Subkingdom	Viridiplantae
Infrakingdom	Streptophyta – land plants
Superdivision	Embryophyta
Division	Tracheophyta – vascular plants, tracheophytes
Subdivision	Spermatophytina – spermatophytes, seed plants, phanérogames
Class	Magnoliopsida
Superorder	Lilianaes – monocots, monocotyledons, monocotyledons
Order	Commelinales
Family	Pontederiaceae – pickerel-weed
Genus	Eichhornia Kunth – water hyacinth, water-hyacinth
Species	<i>Eichhornia crassipes</i> (Mart.) Solms – common water-hyacinth, water hyacinth, floating waterhyacinth, floating water hyacinth, common water hyacinth

2.1.1 Chemical constitution of *Eichhornia crassipes*

Fresh plant contains 95.5% moisture, 0.04% N, 1.0% ash, 0.06% P₂O₅, 0.20% K₂O, 3.5% organic matter. On a zero-moisture basis, it is 75.8% organic matter, 1.5% N, and 24.2% ash. The ash contains 28.7% K₂O, 1.8% Na₂O, 12.8% CaO, 21.0% Cl, and 7.0% P₂O₅. The CP contains, per 100 g, 0.72 g methionine, 4.72 g phenylalanine, 4.32 g threonine, 5.34 g lysine, 4.32 g isoleucine, 0.27 g valine, and 7.2 g leucine (**Matai and Bagchi, 1980**). Water hyacinth roots naturally absorb pollutants, including such toxic chemicals as lead, mercury, and strontium 90 (as well as some organic compounds believed to be carcinogenic) in concentrations 10,000 times that in the surrounding water (**Jafari, 2010; kumar, 2012**).

Phenolic compounds-p-hydroxybenzoic, ferulic, chlorogenic, protocatechuic, vanillic, p-coumaric (**Martyn and Cody, 1983**), and o-hydroxy phenols (**Martyn et al, 1983**), phenolic compounds are detected in the leaves (**Anjana and Matai, 1990; Center and Wright, 1991**). 4-Methylresorcinol, 2-methylresorcinol, catechol, pyrogallol, and genetisic, p-hydroxybenzoic, syringic, vanillic and salicylic acid, Flavonoids-kaempferol,

orientin (**Nyananyo et al, 2007**), quercetin, isovitexin, and kaya flavone (**Lata and Dubey, 2010**) are also present in the shoot and rhizome³, Alkaloids- 18, 19-secoyohimban-19-oic acid, and 16, 17, 20, 21-tetrahydro-16-(hydroxymethyl)-methyl ester (**Shanab et al, 2010; Shanab et al, 2011**), Terpenoids- indole compounds and gibberellins, Sterols such as Campesterol, stigmasterol and sitosterol (**Goswami et al., 1983**), Glycosides and Tannins like compounds, (**Silva et al., 2006**), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, phytol (**Muthunarayanan et al., 2011**) Etc. *E. crassipes* showed the highest contents of calcium (1.51%), magnesium (3,916.67 mg kg⁻¹), manganese (1,233.33 mg kg⁻¹), zinc (81.83 mg kg⁻¹), iron (5,425.00 mg kg⁻¹) and copper (25.83 mg kg⁻¹) (**Silva et al., 2006**).

2.2 Reduced graphene oxide

Graphite oxide was first prepared by Oxford chemist Benjamin C. Brodie in 1859, by treating graphite with a mixture of potassium chlorate and fuming nitric acid. He reported synthesis of "paper-like foils" with 0.05 mm thickness. (https://en.wikipedia.org/wiki/Graphite_oxide). The completely oxidised compound can then be dispersed in a base solution such as water, and graphene oxide is then produced. Reducing graphene oxide is produced by number of ways.

2.2.1 Preparation of Plant based graphene oxide

Graphene oxide was synthesized using the chemical exfoliation method and reduced using *Melissa officinalis* (Melisa) extract. Characterization studies were carried out using UV–Vis, Fourier Transform Infrared Spectroscopy (FTIR), Thermo Gravimetric Analysis (TGA), Zeta Sizer (ZS), X-Ray Powder Diffraction (XRD), Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) images and its cytotoxic and proliferative effects were examined using a minimum essential media elution test and a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. The results showed that RGO–HA (1%) composites are biocompatible and even though they are proliferative at concentrations lower than 25% (**Elif et al., 2017**).

Green synthesis for the reduction of GO was carried out using aqueous leaf extracts of *Paederia foetida* L. The prepared GO and green RGO were characterized by using ultraviolet–visible, Raman and Fourier transform infra-red spectroscopic analysis. The morphology of the green RGO was characterized by transmission electron and scanning electron microscopy. Dynamic light scattering was used for zeta potential measurement and correlated with the morphology of the sheets. Electrical conductivity was also measured to check the extent of reduction of GO to RGO (**Chattopadhyay et al., 2016**).

Bio-synthesis of graphene by a using *Artemisia herba-alba* Asso (AHAA) natural extract. UV–VIS, Raman, XPS spectroscopies and TEM microscopy investigations confirmed the reduction, and the conversion of graphene oxide to few-layered reduced graphene oxide the optical and electrical properties of graphene can be modulated. Hence,

AHAA can be an effective chelating agent to produce graphene sheets (**Khenfouch et al., 2016**)

T. bellirica fruit (pericarp) aqueous extracts as green reducing and stabilizing agent for graphene synthesis from graphene oxide (GO). The synthesized RGO was characterized using UVVisible, XRD, SEM, TEM and FTIR techniques (**Maddinedi et al., 2016**).

Reduced graphene oxide nanosheets were prepared by using **caffeine** as the reductant. The samples of GO, before and after reduction with caffeine have been characterized by X-ray diffraction, Raman, Fourier transform infrared, X-ray Photoelectron spectroscopy, thermogravimetric analysis and transmission electron microscopy, The obtained graphene nanosheets from natural raw graphite are potential biomaterial for many biomedical applications, including nanocarriers for targeting and localized drug delivery, bioimaging and biosensing (**Thu Ha Thi Vu et al., 2015**).

The bioreduction of GRO using *Salvadora persica L. (S. persica L.)* roots (miswak) extract as a bioreductant is reported. The developed eco-friendly method for the reduction of GRO could provide a better substitute for a large-scale production of dispersant-free graphene and graphene-based materials for various applications in both technological and biological fields such as electronics, nanomedicine, and bionic materials (**Khan et al., 2015**).

The preparation of a silver–reduced graphene oxide (Ag–RGO) nanocomposite using *Potamogeton pectinatus (Po)* plant extract is reported. The size, morphology and crystallinity of the as-prepared nanomaterials were studied with an explanation for the role of *Po* in the synthesis. A preliminary antibacterial experiment was developed to ensure the enhanced antibacterial effect of the Ag–RGO nanocomposite. The results indicated that the majority of the silver nanoparticles “AgNPs” were formed in a spherical shape with small sizes ranging from 11 to 20 nm. IR spectroscopy results indicated the role of amine and hydroxyl groups from *Po* in the reduction and capping processes. The preliminary antibacterial examination ensured the enhanced antibacterial effect of the Ag–RGO nanocomposite (**Sedki et al., 2015**).

Water-soluble and cytocompatible graphene using *Ginkgo biloba* extract (GbE) as a reducing and stabilizing agent the biocompatibility effects of graphene in MDA-MB-231 human breast cancer cells is reported. X-ray diffraction studies confirmed the crystalline nature of graphene. SEM was used to investigate the surface morphologies of GO and Gb-rGO. AFM was employed to investigate the morphologies of prepared graphene and the height profile of GO and Gb-rGO. The formation of defects in Gb-rGO was confirmed by Raman spectroscopy. The biocompatibility of the prepared GO and Gb-rGO was investigated using a water-soluble tetrazolium 8 assay on human breast cancer cells. GO exhibited a dose-dependent toxicity, whereas Gb-rGO-treated cells showed significant biocompatibility and increased ALP activity compared to GO (**Gurunathan et al., 2014**)

Reduced graphene oxide (RGO) was prepared from graphite oxide (GO) by using pollen grains (Pgs) of *Peltophorum pterocarpum* as a reducing agent, and studied for its electrochemical behavior. Cyclic voltammetry studies showed the good electrochemical performance of RGO with a maximum specific capacitance of 27.1 F g^{-1} (at a scan rate of 5 mV s^{-1}) (Rahman *et al.*, 2014).

Preparation of graphene (PE-HRG-Ag) via simultaneous reduction of both graphene oxide (GRO) and silver ions using *Pulicaria glutinosa* plant extract (PE) as reducing agent is reported. The preparation of PE-HRG-Ag nanocomposite is monitored by using ultraviolet–visible (UV-Vis) spectroscopy, powder X-ray diffraction (XRD). The as-prepared PE-HRG-Ag nanocomposites display excellent surface-enhanced Raman scattering (SERS) activity, and significantly increased the intensities of the Raman signal of graphene (Abdulhadi *et al.*, 2014).

An ecofriendly process of reduction of graphene oxide using aqueous extract of *Amaranthus dubius* under refluxing method UVVisible spectrophotometer was used to monitor the formation of reduced graphene oxide (AKRGO). The crystallite size of nanographene was confirmed by XRD analysis and Scherrer's formula. FTIR spectral analysis revealed the reduction of graphene oxide using aqueous extract of *Amaranthus dubius*. The morphology of the synthesized graphene was examined by SEM analysis (Firdhouse *et al.*, 2013).

Cherry, Magnolia, Platanus, Persimmon, Pine, Maple, and Ginkgo are compared for their abilities to reduce graphene oxide. The optimized reaction conditions for the reduction of graphene oxide were determined as for Cherry (*Prunus serrulata*), reaction time: 12 h, composition of the reaction mixture: 16.7% v/v of plant leaf extract in total suspension, and temperature: 95°C . The degree of reduction caused by Cherry leaf extract was analyzed by elemental analysis and X-ray photoelectron spectroscopy. The reduction of graphene oxide was also confirmed by ultraviolet-visible spectroscopy, Fourier transform-infrared spectroscopy, Raman spectroscopy, X-ray diffraction, transmission electron microscopy, and thermo gravimetric analysis (Geummi *et al.*, 2013)

2.2.2 Preparation of Single layer graphene oxide

Graphene oxide (GO) films with two-dimensional structure were successfully prepared via the modified Hummer method. Comprehensive characterizations of the properties of GO films were conducted. TEM and DFM analyses showed single and double lamellar layer structure and a thickness of 2~3 nm. X-ray diffraction (XRD) was selected to measure the crystal structure of GO sheet. Fourier-transform infrared spectra analyzer (FT-IR) was used to certify the presence of oxygen-containing functional groups in GO films. UV-VIS spectrometer and TGA analyzer indicated excellent optical response and outstanding thermal stability. Elemental analyzer (EA) and X-ray photoelectron spectroscope (XPS) analyzed the components synthetic material. (Song *et al.*, 2014).

The comparison between two kinds of single-layer reduced graphene oxide (rGO) sheets, obtained by reduction of graphene oxide (GO) with the electrochemical method and hydrazine vapor reduction, referred to as E-rGO and C-rGO, respectively. Although there is no morphology difference between the E-rGO and C-rGO films but the reduction process to obtain the E-rGO and C-rGO films is quite different. In addition, E-rGO shows better electrocatalysis towards dopamine than does C-rGO (**Wang et al., 2012**).

2.3 Gold nano particles

Gold nanoparticles are really small, with a diameter of 5 nm or less, they can be used as a catalyst to help reactions. Researchers attach molecules to gold nanoparticles that are attracted to diseased regions of the body, such as cancer tumours, and other molecules such as therapeutic drug molecules. This enables the functionalized gold nanoparticles to be used to in targeted drug delivery. Another property that gold nanoparticles have is the capability to convert certain wavelengths of light into heat. As with all metals, gold contains electrons that are not tied to a particular atom but free to move throughout the metal. These electrons help to conduct a current when a voltage is applied across the conductor (<http://www.understandingnano.com/gold-nanoparticles.html>).

2.4 Synthesis gold nano particles using plant extract

The synthesis of gold nanoparticles (AuNPs) has been carried out using the root and leaf extracts from *Vetiveria zizanioides* and *Cannabis sativa*, respectively. The synthesized AuNPs were characterized by a peak at 538 nm in the UV-visible spectrum. SEM images revealed that all particles were spherical with a narrow size range of 10–35 nm. Antifungal activity of AuNPs was tested for different fungal pathogens using standard disk diffusion method. The results suggest that the synthesized AuNPs can be useful as effective antifungal agent. It is confirmed that AuNPs are capable of rendering high antifungal efficacy and has a great potential for anti-fungal therapy (**Swain et al., 2016**).

The synthesis and physicochemical investigation of gold nanoparticles using an aqueous extract of *Monotheca buxifolia* (Flac.) is reported. The formation of AuNPs was confirmed by UV–Vis spectroscopy showed an absorption peak at around 540 nm. FTIR was used to identify the chemical composition of gold nanoparticles and Au-capped plant extract. The presence of elemental gold was also confirmed through EDX analysis. SEM analysis of the gold nanoparticles showed that they have a uniform spherical shape with an average size in the range of 70–78 nm. The antioxidant activity of *Monotheca buxifolia* (Flac.) extract and Au-capped with the plant extract was also evaluated using $\text{FeCl}_3/\text{K}_3[\text{Fe}(\text{CN})_6]$ in vitro assay (**Anwar et al., 2016**).

Swertia chirata, a critically endangered medicinal plant has been explored for its reducing ability to synthesize polyshaped gold nanoparticles (AuNP). Characterizing of gold nano particles by UV–Vis spectra showed a plasmon resonance peak at 540 nm. TEM

analysis revealed that the average crystalline size of the particles was 50 nm and they were polyshaped viz. spherical, hexagonal and nanotriangles. XRD analysis confirmed the crystalline nature. The possible mechanism of biosynthesis was predicted by FTIR. The process of AuNP biosynthesis was optimized by response surface methodology (RSM) and maximum biosynthesis was achieved under the optimized condition of 17.24 % leaf extract, pH 4.6, gold chloride concentration 4 mM and temperature 53.61 °C (**Saha et al.,2016**).

The optimization and fabrication of gold nanoparticles (Au-NPs) using *Delonix regia* leaf extract is reported. Ultraviolet–visible spectroscopy analysis showed a surface Plasmon resonance peak for prepared Au-NPs at 542 nm, Transmission electron microscopy analysis showed that the particles were spherical and 4–24 nm in size. Energy dispersive X-ray spectroscopy analysis displayed a 2.2 keV peak corresponding to the pure phase gold nanocrystal. X-ray diffraction proved the fabrication of crystalline Au-NPs with face-centered cubic geometry. Furthermore, ζ potential (–15 mV) and Fourier transform infrared data suggested the role of polar polyphenolic compounds of leaf extract in fabrication and stabilization process. Biofabricated nanoparticles are demonstrated to have catalytic activity for the reduction of toxic nitro-organic pollutant o-nitroaniline (**Dauthal et al., 2016**).

Gold nanoparticles were synthesized using a water extract of *Artemisia capillaris* (AC-AuNPs), and their catalytic activity was evaluated in a 4-nitrophenol reduction reaction in the presence of sodium borohydride. Surface plasmon resonance bands shows band at 534~543 nm. Spherical nanoparticles with an average size of $16.88 \pm 5.47 \sim 29.93 \pm 9.80$ nm were observed by transmission electron microscopy. The face-centered cubic structure of AC-AuNPs was confirmed by high-resolution X-ray diffraction analysis. Based on phytochemical screening and Fourier transform infrared spectra, flavonoids, phenolic compounds, and amino acids present in the extract are suggested to contribute to the reduction of Au ions to AC-AuNPs. The removal of extracts greatly enhanced their catalytic activity by up to 50.4 %. The uses of simple centrifugation can be applied to other metallic nanoparticles that are green synthesized with plant extracts to enhance their catalytic activity (**Lim et al., 2016**).

Synthesis and stabilization of gold nanoparticles formed using aqueous *turnip* leaf extract under ambient conditions the formation of gold nanoparticles was monitored using a UV-Vis spectrophotometer and the maximum absorption peak (λ_{max}) at 535 nm with a visual color change to pinkish-red confirmed the gold nanoparticles. Further characterization was done. The as-synthesized gold nanoparticles showed rapid catalytic reduction of methylene blue (MB) dye to leuco MB in the presence of sodium borohydride (NaBH_4) (**Narayanan et al.,2015**).

Gold nanoparticles (Au-NPs) were synthesized using of the brown marine algae *Sargassum muticum* (*S. muticum*) aqueous extract as both a reductant and a capping

agent. The formation of Au-NPs was confirmed through the presence of an absorption peak at 550 nm using a UV–Vis spectrophotometer. A TEM image showed that the particles are spherical in shape with a mean size of 5.42 ± 1.18 nm. The capping of anionic bio-compounds on the surface of nanoparticles was confirmed by zeta potential measurement (-35.8 mV) and is responsible for the electrostatic stability. The bio-synthesized Au-NPs are expected to have notable applications in pharmaceutical and biomedical applications (**Namvar et al., 2015**).

Green synthesis of gold nanoparticles (AuNPs) has been described utilizing the bark extract of ***Abroma Augusta L.*** and chloroauric acid under very mild reaction conditions. The bark extract acted as a both reducing and stabilizing agent. The catalytic activity of the freshly synthesized gold nanoparticles has been demonstrated for the sodium borohydride reduction of 4-nitrophenol to 4-aminophenol, and the kinetics of the reduction reaction have been studied spectro photometrically (**Das et al., 2015**).

The **black cardamom extract** has been used as a reducing agent for $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$. Fourier transform infrared spectroscopy (FTIR) studies, a tentative mechanism of reduction of Au nanoparticles has also been proposed which includes oxidation of 1, 8-cineole to 2-oxo-1,8-cineole. The effect of pH on the synthesis of Au nanoparticles has been carried out (**Singh et al., 2015**).

Synthesis of gold nanoparticles with bacterium ***Zooglea ramigera*** Biosynthesized gold nanoparticles was spherical in shape of the size range of 4–16 nm (by transmission electron microscopy). X-ray diffraction and specific area electron diffraction shows face centered cubic (FCC) crystalline phase with the crystalline size of 19 nm. Gold nanoparticles showed excellent antibacterial activity against Gram-positive *Staphylococcus aureus*, *Streptococcus pyogenes* and Gram-negative *Pseudomonas aeruginosa*, *Escherichia coli* bacterial pathogens. The gold nanoparticles showed anti-tuberculosis impact in investigation against *Mycobacterium tuberculosis* H37RV. A hypothesis for antibacterial action of gold nanoparticles was also explained (**Srivastava et al., 2015**).

Biosynthesis of gold nanoparticles (AuNPs) has been obtained from ***Pseudomonas aeruginosa*** and ***Rhodopseudomonas capsulata*** bacteria. The important parameter, which controls the size and shape of AuNPs, was pH value. The AuNPs were characterized by UV–Vis whose absorbance measured at 540 nm followed by transmission electron microscopy showed the formation of AuNPs in the range of 20–80 nm in diameter at pH 6.5. Scanning electron microscopy revealed the AuNPs ranging from 50 to 70 nm; Fourier transform infrared spectroscopy confirmed the formation of AuNPs in the range of $4,000\text{--}400\text{ cm}^{-1}$. The results spherical AuNPs in the range of 10–20 nm were observed at pH value of 7 whereas a number of nanoplates were observed at pH 4 (**Singh et al., 2014**).

The gold nanoparticles was synthesized by using ***Hibiscus rosa-sinensis***. The surface Plasmon resonance found at 520 nm confirmed the gold nanoparticles synthesis.

The spherical sized nanoparticles in the size range of 16–30 nm were confirmed by Transmission Electron Microscope (TEM). The stability of the nanoparticles is very well proved in the *in vitro* stability tests. The biochemical like alkaloids and flavonoids play a vital role in the nanoparticles synthesis was identified using the Fourier Transform Infrared Spectroscopy (FTIR). Combining the phytochemical and microwave heating, the rapid synthesis of gold nanoparticles is the novel process for the medically applicable gold nanoparticles production (**Yasmin et al., 2014**).

The ***Magnolia kobus*** plant extract produces a diverse mixture of extracellular gold and silver nanocrystals with a majority of polydispersed spheres; however, there are a significant number of homogeneously sized triangles, pentagons, and hexagons (**Lee et al., 2014**).

The bark extracts of the traditional ayurvedic medicinal plant ***Saraca indica***. The polyphenolic compounds which present in plant acted as the reducing agent as well as the stabilizing agent without any additional capping agent. Surface plasmon resonance, HRTEM, AFM, X-ray diffraction, and FTIR studies have been carried out to characterize the nanoparticles. Gold nanoparticles synthesized were of triangular, tetragonal, pentagonal, hexagonal, and spherical shapes. The synthesized gold nanoparticles have been used as a catalyst for the reduction of 4-nitrophenol to 4-aminophenol at room temperature and the kinetics of the reduction reaction has been studied. In the presence of 0.3 mL stabilized AuNP solution (60 mg L^{-1}), the rate constant was calculated to be 0.29 min^{-1} (**Dash et al., 2014**).

Punica granatum juice has been used for the synthesis of gold nanoparticles (AuNPs) at room temperature under very mild conditions. The AuNPs were characterized by surface plasmon resonance spectroscopy, high resolution transmission electron microscopy, fourier transform infrared spectroscopy and X-ray diffraction studies. Catalytic activity of the synthesized colloidal AuNPs has also been demonstrated. Utilizing the UV-visible data, the catalytic rate constant (k) was calculated to be 0.22 min^{-1} (**Dash et al., 2014**).

Sonochemical synthesis of gold nanoparticles by using ***piper betle leaf broth*** as a reducing and capping agent is reported. The aqueous reaction of medium containing gold nanoparticles showed a peak at 540 nm was studied by UV-Vis spectra. The crystalline structural characteristics of a biomolecules hosted gold nanoparticles were studied by X-ray diffraction. The morphology of nanoparticles was analyzed by scanning electron microscopy. The stoichiometric chemical composition of elemental presence in the medium was determined by energy dispersive spectrum. The presences of biomolecules which are act as capping agents around the nanoparticles were studied by Fourier transform infrared spectroscopy (**Mallikarjuna et al., 2013**).

Natural precursor *Prunus armeniaca* (apricot) fruit extract was used as a reducing agent for the nanoparticle synthesis. The synthesized nanoparticles were characterized. Dose-dependent scavenging activity was observed for Au-NPs and Ag-NPs in both DPPH and ABTS in-vitro assay. 50 % scavenging activity for DPPH were 11.27 and 16.18 mg and for ABTS 3.40 and 7.12 mg with Au-NPs and Ag-NPs, respectively (**Dauthal et al., 2013**).

Biosynthesis of gold nanoparticles using *Padina gymnospora* is reported in literature. The UV–vis spectrum of the aqueous medium containing gold ion showed peak at 527 nm corresponding to the plasmon absorbance of gold nanoparticles. Scanning electron microscopy showed the formation of well-dispersed gold nanoparticles. FTIR spectra of brown alga confirmed that hydroxyl groups present in the algal polysaccharides were involved in the gold bioreduction. AFM analysis showed the results of particle sizes (53–67 nm) and average height of the particle roughness (60.0 nm). X-ray diffraction (XRD) spectrum of the gold nanoparticles exhibited Bragg reflections corresponding to gold nanoparticles. This environment-friendly method of biological gold nanoparticle synthesis can be applied potentially in various products that directly come in contact with the human body, such as cosmetics, and foods and consumer goods, besides medical applications (**Singh et al., 2013**).

Green synthesis of gold nanoparticles has been carried out using the algae extract of *Turbinaria conoides*. The broad surface plasmon resonance band was centered at 520 to 525 nm which indicates polydispersed nanoparticles. Transmission electron microscopy and selected-area electron diffraction analysis show the morphology and crystalline structure of synthesized gold nanoparticles with the size range of 6 to 10 nm. The four strong diffraction peaks were observed by X-ray diffraction; it confirmed the crystalline nature of synthesized gold nanoparticles. The carboxylic, amine, and polyphenolic groups were associated with the algae-assisted synthesized gold nanoparticles which was confirmed using Fourier transform-infrared spectroscopy. Thus, algae-mediated synthesis process of biomedically valuable gold nanoparticles is a one-spot, facile, convenient, large-scaled, and eco-friendly method (**Kumar et al., 2013**).

The leaf extract of *Acacia nilotica* (**Babool**) has been used for the synthesis of gold nanoparticles in water at room temperature under very mild conditions. The gold nanoparticles were characterized by HRTEM, surface plasmon resonance spectroscopy, and X-ray diffraction studies. The synthesized gold nanoparticles have been used as an efficient catalyst for the reduction of 4-nitrophenol to 4-aminophenol in water at room temperature (**Majumdar et al., 2013**).

Aspergillus fumigatus was used for the intracellular synthesis of gold nanoparticles. Stable nanoparticles were produced when an aqueous solution of chloroauric acid (HAuCl₄) was reduced by *A. fumigatus* biomass as the reducing agent. The produced nanoparticles were then characterized by Fourier transform infrared

spectroscopy (FT-IR), scanning electron microscope (SEM), energy dispersive spectroscopy (EDS) and X-ray diffraction spectroscopy (XRD). SEM images of sample revealed that the nanoparticles were spherical, irregularly shaped with indefinite morphology. Biosynthesized gold nanoparticles were in the range of 85.1–210 nm in size. The presence of gold nanoparticle was confirmed by EDS analysis. Crystalline nature and face-centred cubic structure of synthesized gold nanoparticle was confirmed by XRD pattern (**Bathrinarayanan et al., 2013**).

Synthesis of gold nanoparticles (GNP) using the aqueous extract of red tomato (***Lycopersicon esculentum***). This biosynthesized GNP in the presence of sodium dodecyl sulfate has been used as a colorimetric sensor to detect and estimate the pesticide, methyl parathion (**Barman et al., 2013**).

The green synthesis of gold nanometre scale particles using the leaf extract from an indigenous Australian plant ***Eucalyptus macrocarpa*** as both the stabilising agent and the reducing agent is well known. Formation of the gold nanometre sized particles was confirmed and characterised by UV-visible spectroscopy, X-ray diffraction, transmission electron microscopy and field emission scanning electron microscopy. The antibacterial activity of the synthesised gold particles was also quantified using the sensitivity method of Kirby–Bauer (**Poinern et al., 2013**).

The biological synthesis of gold nanoparticles using the culture supernatant of ***Bacillus subtilis*** and its combined antibacterial and antifungal activities with various antibiotics were observed against clinical isolates (**Thirumurugan et al., 2012**).

Gold nanoparticles (NPs) were synthesized using ***Semecarpus anacardium*** leaf extracts. NPs were quantified using UV and ICP-AES analysis. These were characterized using Transmission electron microscopy, Fourier transform infrared spectroscopy and X-ray diffraction. TEM images of the particles formed with green extract, boiled extract and green biomass showed that the particles were of different shapes and sizes (**Raju et al., 2011**).

The extracellular production of Ag and Au nanoparticles was carried out from the leaves of the plants, ***Tridax procumbens* L. (Coat buttons)**, ***Jatropha curcas* L. (Barbados nut)**, ***Calotropis gigantea* L. (Calotropis)**, ***Solanum melongena* L. (Eggplant)**, ***Datura metel* L. (Datura)**, ***Carica papaya* L. (Papaya)** and ***Citrus aurantium* L. (Bitter orange)** by the sunlight exposure method. Among these *T. procumbens*, *J. curcas* and *C. gigantea* plants synthesized <20 nm sized and spherical-shaped Ag particles, whereas *C. papaya*, *D. metel* and *S. melongena* produced <20 nm sized monodispersed Au particles. Qualitative characterization was done by UV–vis spectroscopy and transmission electron microscopy (TEM), respectively. X-ray diffraction (XRD) and X-ray photoelectron spectroscopy (XPS) were used for structural confirmation. Fourier transform infrared spectroscopy (FTIR), provided evidence for the

presence of amino groups, which increased the stability of the synthesized nanoparticles (**Rajasekharreddy et al., 2010**).

Biosynthesis of spherical gold nanoparticles and gold nanoplates was achieved at room temperature and pH 2.8 when cell extract from the metal-reducing bacterium ***Shewanella algae*** was used as both a reducing and shape-controlling agent. The yield of gold nanoplates prepared with *S. algae* extract was four times higher than that prepared with resting cells of *S. algae*. The resulting biogenic gold nanoparticle suspensions showed a large variation in color, ranging from pale pink to purple due to changes in nanoparticle morphology (**Ogi et al., 2010**).

Addition of microwave-exposed aqueous extracellular anti-malignant guava (***Psidium guajava***) leaf extract to the aqueous gold chloride solution yielded stable polyshaped gold nanoparticles of high composition. The formation of nanoparticles was understood from the UV-visible and X-ray diffraction studies. The size and shape analysis was done using field emission scanning electron microscopy, transmission electron microscopy, and atomic force microscopy. Zeta potential experiment shows that the bio-functionalized gold nanoparticles colloidal solution obtained as above will maintain its stability even after 30 weeks of storage (**Raghunandan et al., 2009**).

Persimmon (*Diopyros kaki*) leaf extract is used for the synthesis of bimetallic Au/Ag nanoparticles. UV-visible spectroscopy was monitored formation of Au/Ag nanoparticles. SEM images showed that large Au/Ag particles of 50–500 nm were formed with some cubic structure, while pure Ag particles obtained by reduction of only Ag⁺ ion were smaller with diameter of 15–90 nm and predominantly spherical. The atomic Ag contents of the bimetallic Au/Ag nanoparticles from EDS and XPS analysis were 36 and 71 wt%, respectively, suggesting that bimetallic Au core/Ag shell structure was formed by competitive reduction of Au³⁺ and Ag⁺ ions with Persimmon leaf extract (**Song et al., 2008**).

2.5 Anticancer activity of gold nano particles

Gold nanoparticles (GNPs) were achieved using the ***Albizia amara Roxb*** plant extract. The formation of GNPs was confirmed by a change of color from yellowish green to purple with a characteristic peak at 543 nm. Further, the characterization of the synthesized GNPs showed their crystalline nature, functional groups, size and dispersed shapes, purity and Bragg's reflections of face centered cubic structure. During the DPPH assay, the GNPs and leaf aqueous extract showed the scavenging activity of 74 and 65 %, respectively and their in vitro IC₅₀ inhibitory values ($\mu\text{g ml}^{-1}$) were; 47.77 (HeLa) and 72.28 (Vero). The Gram positive, *Staphylococcus aureus* (MTCC 96) was found to be more sensitive to GNPs (16 mm) than the leaf aqueous extract (12 mm) (**Balasubramani et al., 2016**).

Green synthesis of gold nanoparticles (AuNPs) prepared using water extract from galls of ***Rhus chinensis***. AuNP characterization was performed using ultraviolet-visible

(UV–vis) spectroscopy, transmission electron microscopy, field-emission scanning electron microscopy, and X-ray diffraction analysis. Element composition was detected via energy dispersive X-ray analysis. The sizes of AuNPs ranged from 20 to 40 nm, and they had oval and spherical shapes. The cytotoxic effects against MKN-28 (Adenocarcinoma), Hep3B (Hepatocellular carcinoma), and MG-63 (Osteosarcoma) cells were evaluated using tetrazolium-based assay. The AuNPs induced cytotoxicity in a dose-dependent manner, and morphology upon cell death was differentiated via fluorescent microscopy using 4,6-Diamidino-2-phenylindole dihydrochloride hydrate staining which predicted apoptosis (**Pati et al., 2016**).

Mappia foetida leaves extract is used as bioreductant for the synthesis of gold nanoparticles and their application in the efficient delivery of doxorubicin to human cancer cells. The formation of gold nanoparticles is evident from their characteristic optical absorption at ~560 nm. FCC structure of gold nanoparticles was confirmed by using X-ray diffraction pattern. Fourier transform infrared spectroscopy shows the bioactive molecules from plant extract capped on the surface of gold nanoparticles and conjugation of doxorubicin along with activated folic acid as navigational molecules for targeted drug delivery. Such a conjugation of gold nanoparticles is characterized by their weight loss, ~35–40 %, due to thermal degradation of plant biomass and conjugated drug along with receptor, as observed in thermogravimetric analysis. The spherical shaped gold nanoparticles (Φ 10–20 nm) are observed by field emission scanning electron microscopy and transmission electron microscopy images and the expected elemental composition by energy dispersive X-ray spectroscopy. Gold nanoparticles conjugated with activated folic acid and doxorubicin complex is found to be toxic for human cancer cells viz., MDA-MB-231, HeLa, SiHa and Hep-G2. Furthermore, the amount of drug released was maximum at pH 5.3 (an ambient condition for intravenous cancer drugs) followed by pH 7.2 and pH 6.8 (**Yallappa et al., 2015**).

The biological syntheses of silver and gold nanoparticles from **Gymnema sylvestris** leaf extract and their *in vitro* free radical scavenging efficacy as well as antiproliferative effect in Hep2 cells. The average size of synthesized GYAgNPs and GYAuNPs was found to be 33 and 26 nm, respectively, by DLS particle size analyzer. TEM analysis indicated spherical shape of GYAgNPs and GYAuNPs and in EDX. The cytotoxic effect of GYAgNPs and GYAuNPs in Hep2 cells was examined by MTT assay in which GYAgNPs displayed an IC_{50} value of $121 \mu\text{g ml}^{-1}$, while GYAuNPs produced up to 38 % of inhibition at the maximum concentration of $250 \mu\text{g ml}^{-1}$ used (**Nakkala et al., 2015**).

Gold nanoparticles (AuNPs) were synthesized by sonication using ethanolic leaf extract of **Andrographis paniculata**. Investigation done for optimum parameters for AuNP synthesis and functionalization with polycaprolactone-gelatin (PCL-GL) composites. TEM images showed that nanoparticles were spherical in shape with a size range from 5 to 75

nm. EDX analysis ASQSSs revealed the presence of molecular oxygen and carbon on the surface of AuNPs. The synthesized AuNPs were tested for their effect on HeLa (human cervical cancer) and MCF-7 (human breast cancer) cell lines and found to be nontoxic and biocompatible (**Babu et al., 2012**).

The use of ethanolic extract of *Fagopyrum esculentum* leaves for the synthesis of gold nanoparticles. The synthesized nanoparticles were characterized by UV-visible, transmission electron microscopy (TEM), high resolution TEM (HRTEM) and were found to be spherical, hexagonal and triangular in shape with an average size of 8.3 nm. The crystalline nature of the gold nanoparticles was confirmed from X-ray diffraction (XRD) and selected-area electron diffraction (SAED) patterns. Fourier transform infrared (FT-IR) and energy-dispersive X-ray analysis (EDX) suggested the presence of organic biomolecules on the surface of the gold nanoparticles. Cytotoxicity tests against human HeLa, MCF-7 and IMR-32 cancer cell lines revealed that the gold nanoparticles were non-toxic and thus have potential for use in various biomedical applications (**Babu et al., 2011**).

2.6 Fluorine doped Tin Oxide (FTO)

Spray pyrolysis technique has been used to deposit **Fluorine doped Tin Oxide** (FTO) thin films. Optical constants such as refractive index (n), extinction coefficient (K) and the absorption coefficient of the FTO thin films were determined using spectrophotometric measurement of transmittance, absorbance and reflectance in the spectral range from 172 to 1100nm. The maximum value of transmittance was in the range of 77% to 86%. Furthermore, the effect of annealing temperature and the annealing condition on optical band gap (E_g) was studied. The optical band gap was found to be within the range of 3.50 to 4.0 eV (**Abdullahi et al., 2014**).

Spray pyrolysis deposition (SPD) technique has been employed to prepare large area fluorine-doped tin oxide (FTO), nanocrystalline TiO₂ and catalytic Pt films for dye-sensitized solar cell (DSC) module. The transparent conducting FTO film gave low sheet resistance 8 Ω and average visible light transmittance exceeded 80 %. Large area (15 x 15 cm²) DSC module prepared here shows efficiency 7.4 % at AM-1.5 simulated sun light (**Kaneko et al., 2010**).

2.7 Natural dye based solar cell

Dye sensitized solar cells (DSSC) were fabricated using titanium dioxide nanoparticles sensitized by using extracted from *Acacia concinna* pod it showed solar light to electron conversion efficiencies of 0.69 and 1.17 % respectively. The pre dye treated TiO₂ based DSSC showed 69 % improvement in efficiency when compared to that of conventional DSSC (**Ananth et al., 2016**).

P. amaryllifolius leaves are rich in chlorophyll and widely used as natural colorant to impart deep green colour to food products. A Polyiodide used as a electrolyte solution. Pt coated FTO was used as counter electrode. The photoelectrochemical performance of

the fabricated DSSC based on this dye showed 0.14 mA/cm² short circuit current (J_{sc}) and 0.125 V open circuit voltage (V_{oc}) (Zanan *et al.*,2016).

Betalain pigments extracted from **Red *Bougainvillea glabra*** flower as natural dye sensitizers were fabricated the plasmonic silver nanoparticles (Ag NPs) into the pores of mesoporous TiO₂ electrodes by successive ionic layer adsorption and reaction (SILAR) method. I–V characteristics of the devices were measured by solar simulator (AM1.5 at 100 mW/cm²). The plasmonic enhanced-DSSC giving a short-circuit current density (J_{sc}), fill factor (FF), and power conversion efficiency (PCE) of 1.01 mA cm⁻², 0.77, and 0.27 %, respectively. This development amounts to 50 % efficiency enhancement over the reference DSSC that had a short-circuit current density (J_{sc}), fill factor (FF), and power conversion efficiency (PCE) of 0.7 mA cm⁻², 0.57, and 0.18 %, respectively (Isah *et al.*, 2016).

Anthocyanin (An) and chlorophyll (Chl) dyes have been extracted from **black rice** and **fragrant screwpine (*Pandanus amaryllifolius*)** leaves respectively using methanol as solvent. The photoelectrode prepared by dipping in anthocyanin solution first and then in chlorophyll solution for the same duration showed the best efficiency of 0.81 % with $J_{sc} = 2.64$ mA cm⁻², $V_{oc} = 0.46$ V and $FF = 0.63$ for DSSC (Shah *et al.*, 2016).

DSSC's sensitized by **Trigonella** extracted using water as a solvent exhibited better performance with efficiency of 0.215 %. The performance of the fabricated DSSCs was attempted to enhance by acid treatment of the FTO substrates with HNO₃, H₃PO₄, and H₂SO₄ (Batniji *et al.*, 2016).

Bracts of ***Bougainvillea spectabilis*** and the leaves of ***Euphorbia cotinifolia*** using acidified (0.1 M HCl) distilled water and ethanol separately. The highest open circuit voltage ($V_{oc} = 0.549$ V) and short circuit current density ($J_{sc} = 0.592$ mA/cm²) were obtained respectively under 100 mW/cm² illuminations. The highest power conversation efficiency (η) was 0.175 % (Yazie *et al.*,2016).

Dye-sensitized solar cells were fabricated using natural dyes extracted from **common pear (*Opuntia dillenii*)** and **red tamarind (*Tamarindus indica*)**. Betalain and anthocyanin were identified as the main pigments that sensitize the semiconductor TiO₂ film. The best conversion efficiency of 0.47 % was achieved from betalain dyes and 0.14 % from anthocyanin dye-sensitized solar cell [under standard Air Mass 1.5 illumination (85 mW cm⁻²)]. The mixture of dye (1:1 mixture) adsorbed onto TiO₂ exhibited an efficiency of 0.20 % (Ramamoorthy *et al.*, 2016).

Rutile-phase seagrass-like-arranged TiO₂ nanorods have been sensitized by flowers of ***Sesbania grandiflora***, leaves of ***Camellia sinensis*** and roots of ***Rubia tinctorum***. The sensitized TiO₂ nanorods-based films have been used as photoanode in natural dye-sensitized solar cells. The films were photoelectrochemically active, and the fabricated solar cells had short-circuit photocurrent density (J_{sc}) lying in the range of 3.7–

4.7mAcm⁻². The efficiency of the fabricated natural dye-sensitized solar cells was found to lie in the range of 0.6–1.036 %, respectively (**Akila et al., 2016**).

The Brazilian pigment-rich **Caesalpinia sappan** heartwood extract was used as dye-sensitized solar cells (DSSC) fabricated using pure and pre dye treated TiO₂ nanoparticles sensitized by natural dye showed solar light to electron conversion efficiencies of 1.09 and 1.65 %, respectively. The pre dye treated TiO₂-based DSSC showed 51 % improvement in efficiency when compared to that of conventionally prepared DSSC (**Ananth et al., 2015**).

The ethanolic extract of anthocyanins from **Vitis labrusca grape** has been used in the sensitization of TiO₂ electrodes and production of a Grätzel cell. The prepared Grätzel cell presents a short-circuit current of 0.045 mA/cm², above that of the cell sensitized with commercial N719 dye, which was 0.032 mA/cm². However, the open circuit voltage was 0.293 V, lower than that sensitized with N719 (0.335 V). The fill factor of the anthocyanin sensitized cell was 46.19 %, higher than that of the N719 sensitized cell, 42.72 % (**Szostak et al., 2015**).

The solar cell's was prepared by using **Green Cabbage** tested under an indoor room light, a halogen lamp and direct sunlight. The experimental results greatest photoelectric conversion efficiency (η) of up to 0.1%, an open-circuit voltage (VOC) of 532 mV, and a short-circuit current density (J_{sc}) of 1.2 mA/cm² (**Amadi et al., 2015**).

Solar cell was fabricated using chlorophyll extracted from green leaves of **Chromolaena odorata** were investigated. The nanocrystalline ZrO₂-TiO₂ films were synthesized by the precipitation synthesis. The samples were characterized using X-ray diffraction, UV-vis absorption spectroscopy, Fourier transform infrared spectroscopy and scanning electron microscopy. The photoelectrodes were prepared using ZrO₂-TiO₂ sensitized with the chlorophyll dye and the counter electrodes using reduced graphene oxide. The power was 10 mW cm⁻². The fill factor, Pmax, Jsc and Voc of the cell was calculated to be 38.1%, 9.6 μ W, 0.279 mA cm⁻² and 0.091 V, respectively. The conversion efficiency was obtained to be about 0.1% (**Pai et al., 2015**).

The effect of the **Pedaliium Murex (P. Murex)** leaf extract The open circuit voltage (V_{oc}), short circuit current density (I_{sc}) and efficiency (η) of the constructed DSSC were determined (**Marimuthu et al., 2015**). Extracts from roots of **Beta vulgaris** were used as natural sensitizers in photoelectrochemical solar cells. Applied CeO₂-TiO₂ to natural dye sensitizer solar cells as a photoelectrode. Short-circuit current density (J_{sc}) and open-circuit voltages (V_{oc}) of 9.0 mA cm⁻² and 680 mV, respectively, were obtained, and an effective energy conversion efficiency of 3.5 % was achieved (**Upadhyay et al., 2014**).

Ipomea pescaprae, Imperata cylindrica (L.) Beauv, and Paspalum conjugatum Berg extracts containing quercetin 3-O- β -d-glucofuranoside, graminone B, and chlorophyll a. The dyes have been successfully sensitized the dye-sensitized solar cell. The cells were

illuminated under 100 mW/cm² AM 1.5 condition. The acidic treatments have been successfully improving the cell efficiencies of *I. pescaprae* dye from 0.45 to 0.53 %, *I. cylindrica* dye from 0.44 to 0.48 %, and *P. conjugatum* dye from 0.50 to 0.69 %. Moreover, the addition of coadsorber into *I. pescaprae* dye is able to improve its cell efficiency from 0.53 to 0.55 % with J_{sc} of 3.738 mA/cm², V_{oc} of 0.393 V, and the FF of 0.377. The combination of dyes from *I. pescaprae* with efficiency of 0.27 % and *P. conjugatum* with efficiency of 0.55 % could be improved to be 0.76 % (**Prima et al., 2014**).

The photo electrochemical performance of the DSSC of the main pigment betacyanin obtained by separation and purification from the **beetroot** extract showed that the photo voltage and photocurrent 435 mV, 9.86 mA, respectively. The overall conversion efficiency of nano WO₃ coated TiO₂ dye-sensitized solar cells exhibit a higher conversion efficiency of 2.2% (**Tripathi et al., 2013**).

Nanocrystalline TiO₂ dye-sensitized solar cells have been fabricated using TiO₂ photo electrode sensitized using the extracts of **red rose** and **table rose** as natural sensitizers. The extracts having anthocyanin pigment (pelargonidin, peonidin and cyanidin), which have hydroxyl and carboxylic groups in the molecule can attach effectively to the surface of TiO₂ film. The solar cell constructed using the red rose sensitized TiO₂ photo-electrode exhibited a short-circuit photocurrent of 4.57 mA/cm² and a power conversion efficiency of 0.81 % and that of table rose sensitized TiO₂ photo-electrode exhibited a short circuit photocurrent of 4.23 mA/cm² and a power conversion efficiency of 0.67 % (**Gokilamani et al., 2013**).

Dye sensitized solar cells (DSSC's) were constructed from **black raspberry (Rubus Ideaus)**, **black carrot (Daucuscarota L.)** and **rosella juice (Hibiscus Sabdariffa L.)**. Platinum-coated counter electrode and liquid Iodide/Iodine electrolyte solution were used to fabricate DSSC's. The efficiencies of solar cells produced with black carrot, rosella and black raspberry juice were calculated as 0.25%, 0.16% and 0.16% respectively, under a sunny day (**Tekerek et al., 2011**).

Dye sensitized solar cells (DSSC) were fabricated using **Allamanda cathartic**, **Bougainvillea spectabilis** and **Cosmos sulphureus** dyes obtained from Fijian flowers. The photoanodes were made from electro-phoretically grown *Titanium dioxide* films coated with dyes. DSSCs with *Cosmos sulphureus* exhibited the best efficiency of 0.54% followed by *Allamanda cathartic* (0.40%) and *Bougainvillea spectabilis* (0.38%) (**Narayan et al., 2011**).

2.7.1 Review on DSSC from various e-resources

In order to know the importance of using the synthesized nanoparticles in solar cell applications it was attempted to study the number of literature work available in various e-resources.

Following table shows the number of references taken from the various e-resources focused on solar cell.

Table: 1 Review on DSSC from various e-resources

T- Total number of publications; **A-**no.of articles; **C-**no.of chapters; **R-**no of reference work entry; **P-**Protocol; **B-**Books;**J-**no of journals;**W-**Webpages

S.no	Key words	Sprinker link						Elsevier			
		T	A	C	REW	P	B	A	W	B	J
1	FTO	10,283	5827	4,368	70	18	-	372	4	1	59
2	FTO plate	1336	888	431	11	6	-	19	-	1	18
3	Solar cell	90074	50647	37067	2251	80	29	7060	6930	186	1460
4	Solar cell fabricated using natural dye	1981	1188	710	83	-	-	80	2	-	74
5	Solar cell fabricated using organic dye	2833	1690	1023	119	1	-	256	4	-	169
6	Solar cell fabricated using inorganic dye	1916	1076	747	93	-	-	141	6	1	99
7	Solar cell fabricated using plant extract	1081	320	699	62	-	-	108	85	-	69
8	Solar cell fabricated using graphing	1423	879	475	69	-	-	1690	4	5	28
9	Solar cell fabricated using graphene oxide	1216	744	410	62	-	-	970	4	2	249
10	Solar cell fabricated using plant based reduced graphene oxide	1089	633	394	62	-	-	62	-	-	61
11	Solar cell fabricated using reduced - graphene oxide using water hyacinth	-	-	-	-	-	-	1	-	-	1
12	Solar cell fabricated using nano particle	3513	2383	974	156	-	-	851	3	5	355
13	Solar cell fabricated using natural nano particles	1762	1063	602	97	-	-	335	3	-	185
14	Solar cell fabricated using metal nano	4241	2901	165	175	-	-	822	2	8	300
15	Solar cell fabricated using non metal nano particle	1853	1014	751	88	-	-	227	1	1	205
16	Solar cell fabricated using gold nano particle	1298	644	576	78	-	-	219	1	2	120
17	Solar cell fabricated using natural dye + gold nano particle	441	185	204	22	-	-	41	2	-	35
18	Solar cell fabricated using plant extract + metal	32	261	618	53	-	-	123	78	-	65
19	Solar cell fabricated using plant extract + Inorganic metal	94	136	330	28	-	-	41	2	-	35

20	Solar cell fabricated using plant extract + nano particle	318	118	172	28	-	-	123	78	-	65
21	Making of solar cell using plant extract + gold nano particle	11	3	5	3	-	-	71	9	-	62
22	Solar cell fabrication using water hyacinth plant extract	23	3	19	1	-	-	1	-	-	1
23	Makingsolar cell using graphene + Gold nano particle	421	208	189	24	-	-	106	8	-	92
24	Fabrication of solar cell using grapheneoxide + gold nano particle	320	155	146	19	-	-	51	5	-	43
25	Solar cell fabrication using Reduced graphene oxide + nano particle	603	339	229	35	-	-	179	10	-	144
26	Solar cell fabricated using Reduced garaphene oxide + Gold nano particle	298	138	141	19	-	-	117	10	-	96
27	Making solarcell using plant based Reduced graphene oxide + Gold nano particle	89	32	48	9	-	-	25	-	-	26
28	Solar cell fabrication using water hyacinth reduced graphene oxide + Gold nano particle	-	-	-	-	-	-	1	-	-	1

Analysis of data from the various chemistry resources such as Springer and Elsevier reveals the following:

- The fabrication of solar cell is preferably done by organic dye over the natural dye
- Graphene oxide using solar cell fabrication is higher than that of reduced graphene oxide.
- The work related to the solar cell fabrication with reduced graphene oxide using *Eichhornia crassipes* was found very less in all the resources.

Analysis of the literature search reveals research in solar cell fabrication using *Eichhornia crassipes* and its compounds to be less but more viability of research work of using *Eichhornia crassipes* in solar cell application

MATERIALS AND METHODS

3. Materials and Methods

The study which is aimed to preparing gold nano particles by various methods and preparing reduced graphene oxide using *Eichhornia crassipes (Mart.) Solms* as reducing agent has been carried out. Attempt has been made to explore the pharmacological and photovoltaic application of the prepared nanomaterials.

The materials and methods adopted in this study are given below.

3.1 General

All the chemicals used in the study are of AR grade. Deionized and doubly distilled water were used throughout the study. The chemicals and samples are weighed by using Electronic balance (**Uni Bioc 1987**). Sonication method was used to preparing nanoparticles by using Ultrasonic bath-(Digital Ultrasonic cleaner LMUC series. Microwave method for preparing nano particles from plant extracts were carried out in LG model ECN: MS-1947C/01 of output power Max-1200W microwave oven. 1x1 & 2x2 cm FTO glass plates were used to prepare the solar cell. The current and voltage was measured using Kusam Mecco 405 multimeter. The UV Spectrometer (Biospec-nano (230V)) was used to measuring the absorbance of the nano particles.

SEM analyses were carried out to find the surface morphology of the nanomaterials. Magnetic stirrer (REMI 1MLH) used for stirring involved in the graphene oxide from graphite powder. Refluxing method was carried out for the preparation of reduced graphene oxide. The anti cancer studies were carried out on the reduced particles formed by well plate method. 40 V tungsten lamp was used as a light source for DSSC.

Refluxing apparatus used to prepare the reduced graphene oxide by Hummer's method. The functional group of nano particles was identified by using FTIR (Perkin Elmer) spectroscopy. Centrifuge machine (REMI RM12C) used to separating the solute and precipitate. Hot air Oven (181818 size, 1750W, SL.NO:2/470) was used to produced gold nano particles.

Photography's of instruments



Figure 2: Photograph of Magnetic Stirrer REMI 1MLH



Figure 3: Photograph of 1750 W Hot air Oven



Figure 4: Photograph of Multimeter KUSAM-MECO405



Figure 5: Photograph of Centrifuge machine (REMI RM12C)



Figure 6. Photography of LG model Microwave oven



Figure 7: Photography of Refluxing apparatus

3.2 Preparation of reagents for the study

3.2.1 Preparation of hydrogen peroxide solution

Hydrogen peroxide (30%) was prepared using 30 ml H₂O₂ in 100 ml and stored in brown bottle. This solution was used for the preparation of Graphene oxide.

3.2.2 Preparation of 10% HCl solution

Hydrochloric acid (10%) solution was prepared by dissolving 10ml concentrated hydrochloric acid in 100ml distilled water.

3.2.3 Preparation of Iodine solution

Iodine solution was prepared by dissolving Potassium Iodide and Iodine (2:1) in 4ml distilled water. The two solutions were mixed together and stored in brown bottle for further use.

3.2.4 Purification of ethanol

Ethanol was purified by Distillation as reported earlier (**Helmenstine, 2017**).

3.3 Preparation of stock solutions

3.3.1 Preparation of gold chloride solution

Gold chloride (3 mM) solution was prepared and refrigerated for further use.

3.4 Collection of plant

Eichhornia crassipes (Mart.) Solms was collected from a local water body in Coimbatore

3.4.1 Preparation of aqueous extract by boiling method

Fresh plant of *Eichhornia crassipes* (Mart.) Solms was heated in a beaker with distilled water. Then the hot solution was initially filtered using cotton and then using Whatmann no 42 filter paper then the solutions was closed tightly and it was taken for further studies.

3.5 Phytochemical tests

Preliminary Phytochemical test of the extracts of *E. crassipes* (Mart.) Solms. was carried out as per standard procedure (**Jayanth et al., 2011**).

3.5.1 Test for Alkaloids

1) Mayer's test

A fraction of extract was treated with Mayer's test reagent (1.36 g of mercuric chloride and 5 g of potassium iodide in 100 ml water) and observed for the formation of cream colored precipitate.

2) Wagner's test

A fraction of extract was treated with Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml water) and observed for the formation of reddish brown colour precipitate.

3) Dragendroff's test

A small amount of extract treated with dragendroff's reagent (solution of potassium Bismuth Iodide) and observed the formation of red precipitate.

3.5.2 Test for Flavonoids

1) Sodium hydroxide test

A small amount of extract was treated with aqueous sodium hydroxide and hydrochloric acid, observed for the formation of yellow orange colour.

2) Sulphuric acid test

A fraction of the extract was treated with concentrated H_2SO_4 and observed for the formation of orange colour.

3) Aqueous ammonia test

Extract of water hyacinth treated with aqueous ammonia and observed the formation of yellow orange colour.

3.5.3 Test for Sterols

1) Liebermann-Burchard test

Extract (1ml) was treated with chloroform, acetic anhydride and drops of H_2SO_4 was added and observed for the formation of dark pink or red colour.

2) Salkowski's test

Add 1ml chloroform to the extract and concentrated sulphuric acid added to the mixture and the formation of blue layer in chloroform indicates the presence of sterols.

3.5.4 Test for Terpenoids

1) Liebermann-Burchard test

Extract (1ml) was treated with chloroform, acetic anhydride and drops of H_2SO_4 was added and observed for the formation of dark green colour.

3.5.5 Test for Anthraquinones

1) Borntrager's test

About 50 mg of powdered extract was heated with 10% ferric chloride solution and 1ml concentrated HCl. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia; pink or deep red colourations of aqueous layer indicate the presence of anthraquinone.

3.5.6 Test for Anthocyanin

1) NaOH test

A small amount of extract was treated with 2M NaOH and observed for the formation of blue green colour.

3.5.7 Test for Proteins

1) Ninhydrin test (Aqueous)

The extract was treated with aqueous ninhydrin and observed for the presence of blue colour, indicating the presence of amino acid or purple colour indicating the presence of protein.

3) Biuret test

The extract was heated in distilled water and filtered. The filtrate is treated with 2% copper sulphate solution, to this added 95% ethanol and potassium hydroxide and observed for the formation of pink ethanolic layer.

3.5.8 Test for Phenols

1) Ferric chloride test

The fraction of extract was treated with 5% ferric chloride and observed for the formation of deep blue or black colour.

2) Lead acetate test

The extract was treated with lead acetate solution and observed for the formation of yellow precipitate.

3.5.9 Test for Quinones

A small amount of extract was treated with concentrated HCl and observed for the formation of yellow colour precipitate.

3.5.10 Test for Carbohydrates

1) Molisch's test for Carbohydrates

Few drops of Molisch's reagent were added to each of the portion dissolved in distilled water; this was then followed by addition of 1 ml conc. H_2SO_4 by the side of the test tube. The mixture was then allowed to stand for two minutes and then diluted with 5 ml distilled water. Formation of a red or dull violet colour at the interphase of the two layers was a positive test.

2) Fehling's test for free reducing sugar

About 0.5 g of each extract was dissolved in distilled water and filtered. The filtrate was heated with 5 ml equal volumes of Fehling's solution A and B. Formation of a red precipitate of cuprous oxide was an indication of the presence of reducing sugars.

3.6 Preparation of Graphene oxide (Modified Hummer's method)

Graphene oxide was prepared by modified Hummer's method. Graphite powder (0.5 g) was treated with concentrated sulphuric acid (11.5 ml) in ice-cold condition and stirred for 1/2 h. 1.5g of Potassium permanganate was added gradually over a period of 30 min at temperature $20^\circ C$ for 2 hours. 23 ml doubly distilled water was added to the mixture and maintaining the temperature bellows $100\ C$ for 30 minutes. Finally 30% Hydrogen peroxide (70 ml) was added until the solution turned pale yellow colour. The obtained solution was filtered with 10 % hydrochloric acid to remove the metal ions and

repeated washing with double distilled water to obtain pure graphene oxide. Thus, the obtained graphene oxide was homogenized using an Ultrasonic homogenizer and dried in vacuum to get few layer nanographene.

3.6.1 Preparation of plant extract for reduced graphene oxide

Aqueous extract (100 mg) of *E. Crassipes* was sonicated with 100 ml distilled water at 30^o C for 30 minutes. Then it was filtered using Whatman – 42 filter paper and kept for further study.

3.6.2 Reduction of Graphene oxide using plant extracts (Firdhouse *et al.*, 2014)

Prepared plant extract of *E. Crassipes* is used for reducing the graphene oxide. Graphene oxide (180mg) was sonicated with 360 ml double distilled water for 30 min to obtain a stable suspension. The stable solution was treated with 30ml aqueous extract of *E. Crassipes* and refluxed. The temperature is maintained at 90^oC - 100^oC for 6 hours. The brown colour solution changed to black colour indicating the complete the reduction reaction. Then it was again sonicating for one hour in sonic bath, after sonication solution it was filtered using Whatman -42 filter paper then dried.

3.7 Characterization of Reduced Graphene oxide

The reduced graphene oxide was characterized by using the following spectroscopy techniques.

 UV–Vis spectroscopy

 Fourier Transform Infrared Spectroscopy analysis

 Scanning Electron Microscopy analysis

3.7.1 UV- Visible spectroscopy

UV–Vis spectral analysis was performed using UV-Visible spectrometer (Biospec-nano (230V)).UV-Visible spectroscopy techniques quantify the light that is absorbed and scattered by a sample. The reduction of graphene oxide was also monitored by UV- Visible spectroscopy and it is a basic tool to confirm the formation of reduced graphene oxide.

3.7.2 Fourier Transform Infrared Spectroscopy

FTIR provides an excellent means to visualize the chemical composition of different wheat varieties and it is very quick, reliable and cheaper analytical technique non destructive technique, provides precise measurement which requires no external calibration, increases speed, collecting a scan every second has minimum sample preparation (**Amir *et al.*, 2013**).FTIR spectral measurements were analyzed with the resolution of 0.2 cm⁻¹ for reduced graphene oxide

3.7.3 Scanning Electron Microscopy (SEM)

Electron Microscopes are scientific instruments beam of high-energy electrons to generate a variety of signals electrons to examine objects on a very fine scale. This examination can yield information about the topography (surface features of an object),





morphology (shape and size of the particles making up the object), composition (the elements and compounds that the object is composed of and the relative amounts of them) and crystallographic information (how the atoms are arranged in the object) (**Voutou and Stefanaki, 2008**). Scanning Electron Microscopy uses a focused at the surface of solid specimens. A two-dimensional image is generated that displays spatial variations in properties including chemical characterization, texture and orientation of materials (<https://www.labtesting.com/services/materials-testing/metallurgicaltesting/sem-analysis/>). SEM analysis was taken to finding the surface of the reduced graphene oxide.

3.8 Preparation of the extract for gold nano preparation

Aqueous extract of *E. Crassipes* (1g) was sonicated with 100 ml distilled water at 30° C for 30 min. Then the extract filtered using Whatmann no 42 filter paper. The filtered solution was taken for preparation of gold nano particles.

3.8.1 Synthesis of gold nano particles using plant extract

The gold nano particles using *E. crassipes* plant extract was synthesized by using following methods as per the procedure reported (**Firdhouse et al., 2013**).

-  Room temperature method
-  High temperature method (Hot Air Oven method)
-  Microwave method
-  Sonication method

3.8.2 Synthesis of gold nano particles by room temperature method

In this room temperature method 1ml Gold chloride was used with 1.5ml plant extract. The formation of gold nano particles was confirmed by the visible colour change of the mixture from pale yellow to wine red colour. UV analysis was carried out to confirm the formation of gold nanoparticles.

3.8.3 Synthesis of gold nano particles by sonication

In sonication method, gold chloride 1ml treated with 1.5ml plant extract and the experiment carried out in Ultra – sonication bath (PCI Ultrasonics (1H)).The formation of nanoparticles was confirmed by the colour change and recording the corresponding UV absorbance.

3.8.4 Synthesis of gold nano particles by microwave method

Gold chloride (1ml) and plant extract (1.5ml) were micro waved in an oven and the formation of gold nanoparticles confirmed by the colour change and recording the UV absorbance.

3.8.5 Synthesis of gold nano particles by Oven heating method

The gold nano particles were synthesized by treating various concentrations 1ml, 1.5ml, 2ml, 2.5ml and 3ml of *E. crassipes* aqueous extract with a fixed volume of gold chloride solution (1ml) respectively and the formation of gold nano particles was confirmed

by the colour changed from yellow to wine red colour and the corresponding UV absorbance.

3.9 Characterization of Gold nanoparticles

3.9.1 UV-visible spectrophotometer

Formation of gold nano particles from various methods was analyzed using UV-visible spectrophotometer as it is the first tool to confirming the formation of gold nano particles.

3.9.2 Fourier Transform Infrared Spectroscopy (FTIR)

Prepared gold nano particles by Hot Air Oven method was centrifuged and the residue was subject to FTIR analysis.

3.10 Analyzing the application of synthesized nanoparticles

Application of prepared gold nano particles and reduced graphene oxide using water hyacinth was carried out in the field of Pharmacology and energy related applications i.e.for the fabrication of solar cell.

3.10.1 Preparation of synthesized nanoparticles for anticancer studies.

The synthesized nano particles were tested for anti-cancer efficiency. 50µl, 100 µl, 150 µl, 200 µl, 250 µl concentrations of synthesized nano particles were prepared and used. MTT assay was adopted to study the cytotoxicity and cytoviability of the nanomaterial.

3.11 Pharmacological analysis of application of synthesized graphene and gold nanoparticles

3.11.1 MTT assay for determination of anticancer efficacy of the synthesized nanoparticles (Babu *et al.*, 2011).

3.11.2 Cell line

MCF-7 (Human breast cancer) cells were maintained in the Minimal Essential Medium (MEM) containing 1.0 mmol/L sodium pyruvate, 0.1 m mol/L nonessential amino acids, 1.5 g/L sodium bicarbonate, 2 mmol/L L-glutamine supplemented with 10 % FBS (heat inactivated) and 1 % antibiotic-antimycotic solution (1000 U/mL penicillin G, 10 mg/mL streptomycin sulphate, 5 mg/mL gentamycin, and 25µg/mL amphotericin B). The cells were cultured at 37°C in a humidified incubator (Heal Force, HF 160 W, China) supplemented with 5%CO₂. The statistical software SPSS version 17.0 was used for the analysis. *P* value <0.01 was considered significant.

3.11.3 Cell treatment procedure

To examine the cytotoxicity of prepared compounds, monocultures of the MCF-7 cell lines were incubated with increasing concentrations of filter (0.2 micron) sterilized compounds for 24 h and the cell viability was estimated by MTT dye conversion assay. Cells not exposed to compounds were taken as control. For MTT assay MCF-7 cell were seeded (1104) in a 96-well plate (Cell Bind, Corning). After 24 h of growth, the medium was

replaced with the serum free medium that contained varied concentrations of prepared compounds (from 50 to 250 µg/ µl). After 24 h of treatment the media was removed and cells were washed with phosphate-buffered saline (PBS, 0.01 mol/L, pH = 7.2). All the in vitro experiments were done in triplicate, and the experiments were repeated at least thrice.

3.11.4 MTT assay

3-[4, 5-dimethylthiazol-2-yl] 2, 5- diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate dehydrogenase, cleaves the tetrazolium ring, formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells.

After 48 h of incubation, 15µL of MTT (5mg/mL) in phosphate buffered saline (PBS) was added to each well and incubated at 37°C for 4h. The medium with MTT was then discarded and the formed formazan crystals were solubilised in 100µL of DMSO and then measured the absorbance at 570 nm using micro plate reader the percentage cell viability was then calculated with respect to control as follows:

$$\% \text{ Cell viability} = \text{OD value of experimental sample} / \text{OD value of experimental control} \times 100$$

3.12 Analysis of nano particles in solar cell application

The application of prepared gold nano particles, reduced graphene oxide, its composite and water hyacinth plant extract in different combination in Dye sensitizing solar cell applications has been analysed.

3.12.1 Making positive electrode for DSSC

Dye sensitized solar cell was created by adopting the following procedure.

Initially the two equal- sized (2x2 cm) FTO Glass plate was washed with distilled ethanol and dried. After the washing the plates were handled only at the edges. The conductive side of the plate was identified by using multimeter which in the resistance value. This conductive side was used for coating process. Conductive side of the one plate faces up. A transparent tape was applied on the four sides of the plate to overlap 1 millimeter of the edges to preventing the overflow of the coated materials on the plate. Titanium dioxide was ground for 30 minutes in mortar using piston to make a paste and coated on the glass plate uniformly. Dried the glass plate on a hot plate for 30 minutes. The conductivity was checked using multimeter.

3.12.2 Making negative electrode for DSSC

Another glass plate was tested to find the conductivity side and a thin carbon coating was applied on the conductive side uniformly by using graphite pencil.

3.12.3 Coating the glass plate by using synthesized compounds

Titanium di oxide coated plate was prepared (similar procedure adopted in 3.12.1). Gold nano particles, reduced graphene oxide and composite of gold nano particles with reduced graphene oxide and *E.crassipes* extract were used in fabricating a solar cell by coating these nanomaterials onto the pre-prepared FTO plate. After the coating conductivity was checked using multimeter.

3.12.4 Making the Dye Sensitizing Solar cell

A drop of freshly prepared Iodine solution was applied to the carbon coated plate. The carbon coated plate was placed on the titanium di oxide coatings touching in such manner so that the plates are slightly offset and allowed the iodine solution to soak through the plate coatings so they are covered completely. When the cell is exposed to the light source the cation relays its positive charge from TiO_2 plate to an iodide ion in solution and restores the dye to its original state. Current is generated when the electrons in TiO_2 move through an external circuit and recombine with the oxidized iodide species at the counter electrode. An alligator clip was attached to the sides of the two coated plates.

3.12.5 Calculating the efficiency of solar cell

- ✚ The black wire of the multimeter was connected to the clips which were connected to the exposed titanium dioxide coating. This plate serves as the negative electrode or cathode of the solar cell.
- ✚ The red wire of the multimeter was connected to the clip which was connected to the exposed carbon coating. This portion serves as the positive electrode, or anode of the solar cell.
- ✚ The solar cell was exposed to the sun light and tungsten lamps with the cathode facing the light sources.
- ✚ The current and voltage generated by the solar cell was measured by using Kusam Meco 405 multimeter.
- ✚ The efficiency of the solar cell by using the formula

$$\text{Efficiency} = \text{Jsc} \cdot \text{FF} \cdot \text{Voc} / \text{Pin} \cdot \text{A}$$

RESULTS AND
DISCUSSION

4. RESULTS AND DISCUSSION

“Do something new and expect new result” - Sunday Adelaja

The results and discussion of the study entitled “**Bio fabrication of gold and graphene nano particles – Anticancer & Solar cell application**” are reported in the following pages.

The applications of gold nano particles and reduced graphene oxide were confirmed from the review of past literature, following that the synthesis of these two compounds were done and confirmations were made in this study, and their applications were analyzed. The systematic output of the study is given below.

4.1 Phytochemical assessment of *Eichhornia crassipes*

Preliminary Phytochemical screening of the extracts and solvent fractionates of aqueous extracts of *Eichhornia crassipes* was carried out and the results of the corresponding metabolites are tabulated (**Table 2**).

Table 2: Results of Phytochemical assessment of *Eichhornia crassipes*

S.No.	Aqueous extract
1.Alkaloids	
a. Meyers test	+
b.Wagner’s test	+
c.Dragentroff’s test	-
2.Flavonoids	
a.NaOH Test	-
b. H ₂ SO ₄ test	+
c.Aqueous ammonia test	-
3.Sterols	
a.Liebermann-Burchard test	+
b.Salkowshi test	+
4.Terpenoids	
a.Liebermann-Burchard test	-

5. Anthroquinone	
a. Borntrager's test	-
6. Anthocyanins	
a. NaOH test	-
7. Proteins	
a. Ninhydrin test	-
b. Biuret test	-
8. Phenols	
a. Ferric chloride test	-
b. Libermann's test	-
c. Lead acetate	-
9. Quinones	
a. HCl test	-
10. Carbohydrates	
a. Molisch test	+
b. Fehling's test	+

Various metabolites such as Alkaloids, Flavonoids, Sterols, Anthroquinone, Quinones, and Carbohydrates are present in *Eichhornia crassipes* showed in **table 2**.

The presence of the aforesaid secondary metabolites and also reducing sugar is anticipated to play a role in the synthesis of gold nanoparticles and graphene oxide.

Photography of Phytochemical assessment of *Eichhornia*



Figure 8: Photography of Phytochemical assessment of *Eichhornia crassipes*

Secondary metabolites like Alkaloids and flavonoids play a major role in pharmacological activity. Alkaloid (18, 19-Seco-15 beta-yohimb) was shown to exert potent antibacterial, antifungal and moderate antifungal activities (**Sanaa et al., 2010**). Flavonoids are a group of polyphenolic substances present in *Eichhornia crassipes* plants and are responsible for various biochemical and antimicrobial activities. They exert their antioxidant activity via radical scavenging, metal ion chelation, and membrane protective efficiency (**Kumar et al., 2014**). Terpenoids, polyphenols, sugars, alkaloids, phenolic acids, and proteins, play an important role in the bioreduction of metal ions, yielding nanoparticles. Alkaloids and flavonoids play a vital role in the nanoparticles synthesis (**Yasmin et al., 2014**).

Terpenoids are a class of diverse organic polymers synthesized in plants from five-carbon isoprene units, which display strong antioxidant activity (**Shankar et al., 2003**). Terpenoids play a key role in the transformation of silver ions into nanoparticles in reactions using extracts from geranium leaves. Eugenol, the main terpenoid of *Cinnamomum zeylanisum* (cinnamon) extracts, was found to play the principal role in the bioreduction of HAuCl_4 and AgNO_3 to nanoparticles (**Singh et al., 2010**).

Flavonoids are a large group of poly phenolic compounds that comprise several classes: anthocyanins, isoflavonoids, flavonols, chalcones, flavones, and flavanones, which can actively chelate and reduce metal ions into nanoparticles. Flavonoids contain various functional groups capable of nanoparticle formation. It has been postulated that the tautomeric transformations of flavonoids from the enol-form to the keto-form may release a reactive hydrogen atom that can reduce metal ions to form nanoparticles.

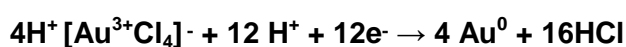
Ocimum basilicum (sweet basil) extracts it is the transformation of flavonoids luteolin and rosmarinic acid from the enol- to the keto-form that plays a key role in the formation of silver nanoparticles from Ag ions (**Ahmad et al., 2010**). Moreover, the internal mechanism of the conversion of ketones to carboxylic acids in flavonoids is likely to be involved in Au³⁺ ion reduction. Isolated flavonoids and flavonoid glycosides have the ability to induce the formation of metal nanoparticles.

Apiin (apigenin glycoside) was extracted from *Lawsonia inermis* (lawsonite thornless, henna) and used for the synthesis of anisotropic gold and quasi-spherical silver nanoparticles with an average size of 21–30 nm (**Kasthuri et al., 2009**).

Amino acids were found to differ in their ability to bind metal ions and to reduce them. Amino acids such as lysine, cysteine, arginine, and methionine are capable of binding silver ions (**Gruen et al., 1975**). Aspartate can reduce tetrachloroauric acid to form nanoparticles (**Manda et al., 2002**).

Protein molecules facilitating the formation of nanoparticles from metal ions display high reducing activity and high potential for attracting metal ions to the regions of amolecule that are responsible for reduction, but that their chelating activity is not excessive (**Glusker et al., 1999**).

Mechanism of the formation of gold nano



Gold chloride solution



Water hyacinth aqueous extract



4.2 Synthesis of gold nano particles using plant extract

The gold nano particles using *E. crassipes* plant extract was synthesized by using following methods such as

- Room temperature method
- High temperature method (**Hot Air Oven method**)
- Microwave method
- Sonication method

The results of the of the synthesis work is given below

4.2.1 Synthesis of gold nano particles by room temperature method

The aqueous extract of *E. Crassipes* (1.5ml) treated with gold chloride (1ml) solution and the colour of the mixture changed from yellow to wine red colour (**Figure.9**).

The gold nano particles were formed within 8 hours. The room temperature maintained at 32°C.

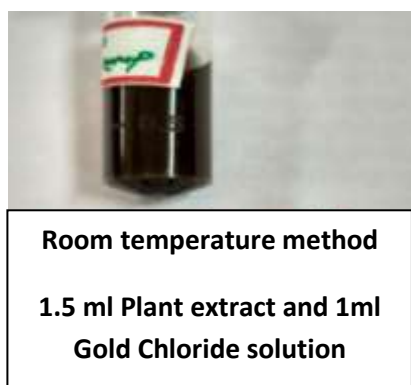


Figure:-9 photography of synthesized gold nano particles using *E. Crassipes* at room temperature method

The formation of gold nano particles were confirmed by UV absorption. The absorbance obtained at 530 nm range (**Figure10**).

No. : 50
 Sample Name : WHAU ROOM TEMP
 Measurement Mode : Photometric
 Wavelength(nm) : 220 250 320 370 430 500 600 780
 DateTime : 17/04/04 12:47:23

OD220 : 34.276
 OD250 : 23.372
 OD320 : 11.249
 OD370 : 6.062
 OD430 : 3.399
 OD500 : 1.906
 OD600 : 1.426
 OD780 : 0.438

Item	Result
Pathlength (mm)	0.189
Dilution	1.000

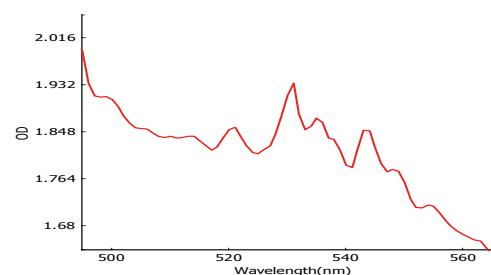


Figure 10: - UV-Visible spectrum of gold nano particles prepared at room temperature

The formation of gold nanoparticles was investigated by UV-visible Spectrophotometer. The Surface Plasmon Resonance band appears at 540nm and peak intensity increases as concentration of the gold chloride increases (**Firdhouse et al., 2013**).

The appearance of the lower color confirms the formation of gold nanoparticles in the reaction mixture and efficient reduction of the Au^{3+} to Au^0 . Colored solution allowed measuring the absorbance against distinct wavelength to confirm the formation of AuNPs. A gradual increase in the intensity of absorbance band without any shift with increasing time from spectra indicates the slow reduction of Au^{3+} to Au^0 (**Bhau et al., 2015**).

The red wine color of the prepared colloidal GNPs solution is a result of absorbing specific parts of the visible light spectrum, mainly at the surface plasmon resonance which corresponds to the green visible light. However, what appears for us is the complementary color which is red wine. The red wine color may be considered as a pointer of the quality of the prepared sample (**Alzoubi et al., 2015**).

In the present the wine red nanoparticles were formed in 8 hours. In order to reduce the time required for synthesis of gold nanoparticles attempts were made to utilize other methods too.

4.2.2 Synthesis of gold nano particles by sonication method

A conventional ultrasonic bath may become a simple apparatus was used without an additional heater or magnetic stirrer under atmospheric conditions to examine the feasibility of forming aqueous GNPs. Gold nano particles using *Eichhornia crassipes* plant extract were prepared using ultra sonic bath and the formation of gold nano particles was confirmed by the colour change from yellow solution to wine red colour (**Figure11**).

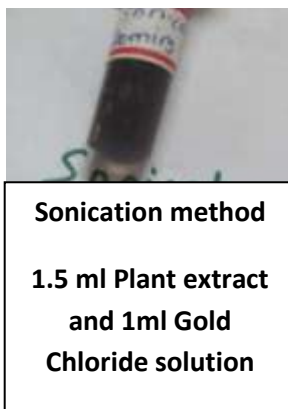


Figure: 11 Synthesized gold nano particles using *E. Crassipes* prepared by sonication method

Nano gold was formed within 10 minutes and formation of gold nano particles was confirmed by UV-Visible spectrum. The UV absorbance was obtained in 530 nm is given in Figure 12.

No. 54
 Sample Name : WHAU SONICATION
 Measurement Mode : Photometric
 Wavelength(nm) : 220 250 320 370 430 500 600 780
 DateTime : 17/04/04 12:56:01

OD220 : 16.275
 OD250 : 10.271
 OD320 : 5.019
 OD370 : 2.834
 OD430 : 1.651
 OD500 : 1.017
 OD600 : 0.700
 OD780 : -0.219

Item	Result
Pathlength (mm)	0.189
Dilution	1.000

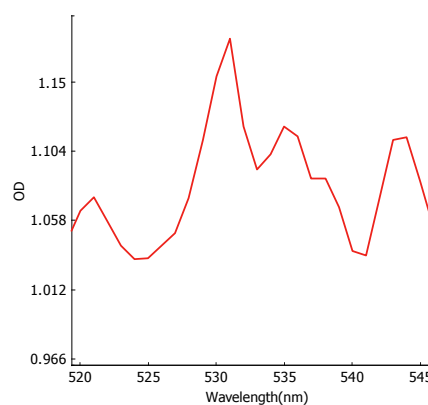


Figure 12: - UV-Visible spectrum of gold nano particles prepared by sonication method

4.2.3 Synthesis gold nano particles using E. Crassipes by microwave method

Microwave method is the most easy and less time consuming method for the preparation of gold nano particles. The formation of gold nano particles was prepared and confirmed by the colour change from yellow to wine red colour (**figure 13**).



Microwave method

**1.5 ml Plant extract
and 1ml Gold
Chloride solution**

Figure13: Synthesised gold nano particles using *E. Crassipes* prepared by microwave method

In 1986 the use of microwaves was first reported by Gedye (**Gedye *et al.*, 1986**) for rapid organic synthesis, observing the advantages of this technique such as fast heating and reaction completion (**Gedye *et al.*, 1991; Tierney *et al.*, 2005**).

This technique has been applied now to develop an alternate methodology to synthesize metallic nanoparticles at relatively short times, allowing a good control of size distribution, and of great importance, it does not require necessarily the use of additional reducing agents (**Hernandez *et al.*, 2010**).

Reduction takes place during the interaction of microwave radiation with reagents in the reaction system forming reducing species *in situ* without the need of mixing other chemicals as reducing agents, thus eliminating additional contamination sources. In this microwave approach, thermal and non-thermal effects can take place, where an increase of thermal energy occurs due to absorption of microwaves as a function of the dielectric properties of the irradiated molecules from chemicals in the reaction system. Solvents can reach temperatures above their boiling points as superheated liquids. Based on this, a kinetic or thermodynamic product can be designe (**Galema *et al.*, 1997**) in this process the reaction is controlled through the setting of temperature, power and time of reaction.

The gold nano particles was formed within 45 seconds by microwave heating. The UV absorbance was obtained in 520-535 nm range and the spectrum of the gold nano particles given in (figure 14)

No. : 53
 Sample Name : WHAU MICROWAVE
 Measurement Mode : Photometric
 Wavelength(nm) : 220 250 320 370 430 500 600 780
 DateTime : 17/04/04 12:53:27

OD220 : 20.009
 OD250 : 12.572
 OD320 : 6.535
 OD370 : 3.869
 OD430 : 2.383
 OD500 : 1.524
 OD600 : 1.095
 OD780 : 0.121

Item	Result
Pathlength (mm)	0.189
Dilution	1.000

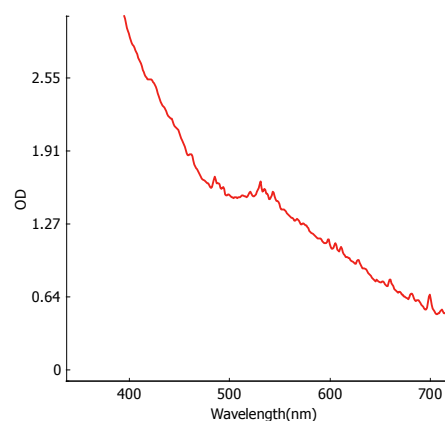


Figure 14: - UV-Visible spectrum of gold nano particles prepared by microwave method

4.2.4 Synthesis gold nano particles using *E. Crassipes* by Hot Air Oven heating method

The main advantages of hot air oven such as sterilisation of those substances which gets spoiled during moist heat sterilization the method employed used to produce gold nano particles. The gold nano particles were synthesized by treating various concentrations viz 1ml, 1.5ml, 2ml, 2.5ml and 3ml of *E. crassipes aqueous* extract with a fixed volume of gold chloride solution (1ml) respectively and the formation of gold nano particles was confirmed by the colour changed from yellow to wine red colour (**figure 15**).



Figure15: Gold nano particles synthesized prepared by hot air oven method

The time required for the formation of gold nano particles using *Eichhornia crassipes* by hot air oven method is given in **tables 3 and 4**.

Table 3. Various concentrations of *Eichhornia crassipes* with constant ratio of gold chloride

Concentration of WH (ml)	Concentration of AuCl ₃ (ml)	Time (min)	Colour of the nanoparticle formed
1	1	5	Wine red
1.5	1	10	Wine red
2	1	15	Wine red
2.5	1	26	Wine red
3	1	32	Wine red

Table 4. Various concentration gold chloride of with constant ratio of *Eichhornia crassipes*

Concentration of WH (ml)	Concentration of AuCl ₃ (ml)	Time (min)	Colour of the nanoparticles formed
1	1.5	25	Wine red
1	2.5	35	Dark blue

The above tables show that as the plant concentration increases the time taken for the formation of gold nano particles was increased from 5-32 minutes, similarly increasing the concentration of gold chloride solution time of formation of gold nano particles

increased. The plant metabolites act as capping agents for the reduction of gold chloride and hence equal quantity of both gold chloride and plant extract are solicited. The UV absorbance for the synthesized gold nano particles gives absorption bands in the region 510-535 nm range.

No. 22
 Sample Name : WHAU A 1+1
 Measurement Mode : Photometric
 Wavelength(nm) : 220 250 320 370 430 500 600 780
 DateTime : 17/04/04 11:52:22

OD220 : 14.416
 OD250 : 8.688
 OD320 : 4.187
 OD370 : 2.277
 OD430 : 1.337
 OD500 : 0.826
 OD600 : 0.658
 OD780 : 0.138

Item	Result
Pathlength (mm)	0.189
Dilution	1.000

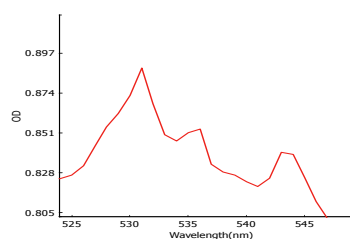


Figure.16 UV – Visible spectrum of extract and AuCl₃ in ratio 1:1

No. 14
 Sample Name : WHAU 1.5+1
 Measurement Mode : Photometric
 Wavelength(nm) : 220 250 320 370 430 500 600 780
 DateTime : 17/04/04 11:38:46

OD220 : 21.747
 OD250 : 13.734
 OD320 : 7.288
 OD370 : 4.271
 OD430 : 2.710
 OD500 : 2.058
 OD600 : 1.855
 OD780 : 0.751

Item	Result
Pathlength (mm)	0.189
Dilution	1.000

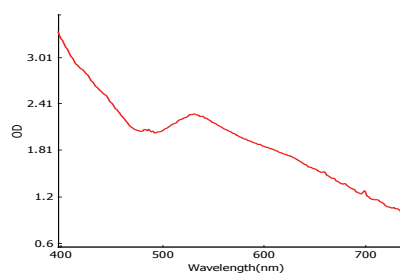


Figure.17 UV – Visible spectrum of Plant extract and AuCl₃ in ratio 1.5:1

No. 24
 Sample Name : WHAU C 2+1
 Measurement Mode : Photometric
 Wavelength(nm) : 220 250 320 370 430 500 600 780
 DateTime : 17/04/04 11:56:11

OD220 : 12.258
 OD250 : 8.111
 OD320 : 4.325
 OD370 : 2.661
 OD430 : 1.749
 OD500 : 1.248
 OD600 : 1.164
 OD780 : 0.019

Item	Result
Pathlength (mm)	0.189
Dilution	1.000

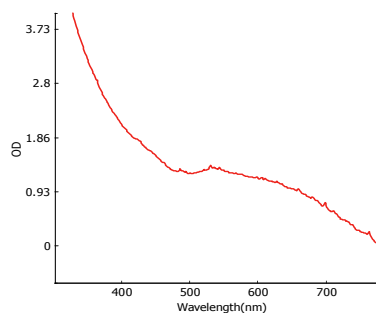


Figure.18 UV – Visible spectrum of Plant extract and AuCl₃ in ratio 2:1

No. 25
 Sample Name : WHAU D 2.5+1
 Measurement Mode : Photometric
 Wavelength(nm) : 220 250 320 370 430 500 600 780
 DateTime : 17/04/04 11:59:38

OD220 : 11.585
 OD250 : 7.972
 OD320 : 4.327
 OD370 : 2.580
 OD430 : 1.616
 OD500 : 1.093
 OD600 : 0.727
 OD780 : -0.178

Item	Result
Pathlength (mm)	0.189
Dilution	1.000

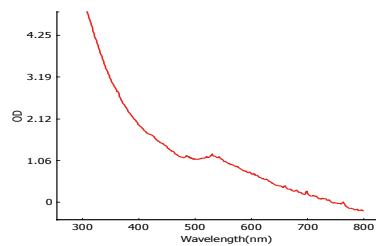


Figure.19 UV – Visible spectrum of Plant extract and AuCl₃ in ratio 2.5:1

No. 30
 Sample Name : WHAU E 3+1
 Measurement Mode : Photometric
 Wavelength(nm) : 220 250 320 370 430 500 600 780
 DateTime : 17/04/04 12:09:03

OD220 : 10.869
 OD250 : 7.484
 OD320 : 3.956
 OD370 : 2.326
 OD430 : 1.434
 OD500 : 0.923
 OD600 : 0.552
 OD780 : -0.275

Item	Result
Pathlength (mm)	0.189
Dilution	1.000

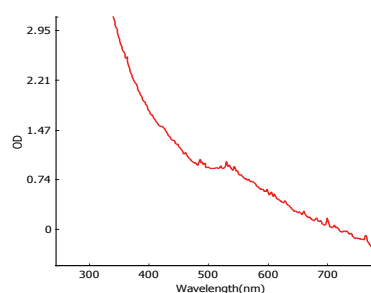


Figure.20 UV – Visible spectrum of Plant extract and AuCl₃ in ratio 3:1

No. 40
 Sample Name : WHAU G 1+1.5
 Measurement Mode : Photometric
 Wavelength(nm) : 220 250 320 370 430 500 600 780
 DateTime : 17/04/04 12:25:12

OD220 : 28.984
 OD250 : 18.001
 OD320 : 9.373
 OD370 : 5.792
 OD430 : 4.105
 OD500 : 3.402
 OD600 : 3.530
 OD780 : 2.244

Item	Result
Pathlength (mm)	0.189
Dilution	1.000

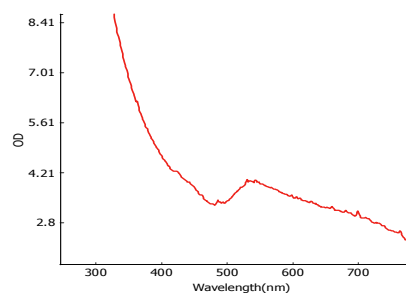


Figure.21 UV – Visible spectrum of AuCl₃ and Plant extract in ratio 1.5:1

No. 35
 Sample Name : WHAU F 1+2.5
 Measurement Mode : Photometric
 Wavelength(nm) : 220 250 320 370 430 500 600 780
 DateTime : 17/04/04 12:17:39

OD220 : 31.656
 OD250 : 15.919
 OD320 : 7.309
 OD370 : 4.200
 OD430 : 2.847
 OD500 : 2.086
 OD600 : 2.211
 OD780 : 1.401

Item	Result
Pathlength (mm)	0.189
Dilution	1.000

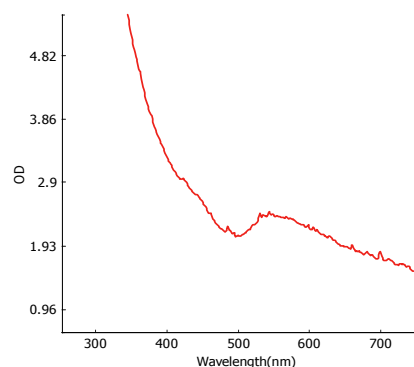


Figure.22 UV – Visible spectrum of AuCl₃ and Plant extract in ratio 2.5:1

4.3 Synthesis of reduced graphene oxide using *Eichhornia crassipes*

Reduced graphene oxide was prepared using the aqueous extract of *Eichhornia crassipes* by refluxing method. The extract was used as reductant. The completion of the reduction reaction was indicated by the brown colour solution changing to black colour (**figure23**) and also confirmed in the next step through UV absorbance analysis.



Figure 23: Photography of synthesized reduced graphene oxide using *Eichhornia crassipes* aqueous extract

The UV absorbance spectrum for synthesized reduced graphene oxide using *Eichhornia crassipes* gives the band at 262 nm.

No. 44
 Sample Name : WHRG I
 Measurement Mode : Photometric
 Wavelength(nm) : 220 250 320 370 430 500 600 780
 DateTime : 17/04/04 12:34:40

OD220 : 1.764
 OD250 : 2.022
 OD320 : 1.433
 OD370 : 1.250
 OD430 : 1.080
 OD500 : 0.811
 OD600 : 0.563
 OD780 : -0.091

Item	Result
Pathlength (mm)	0.189
Dilution	1.000

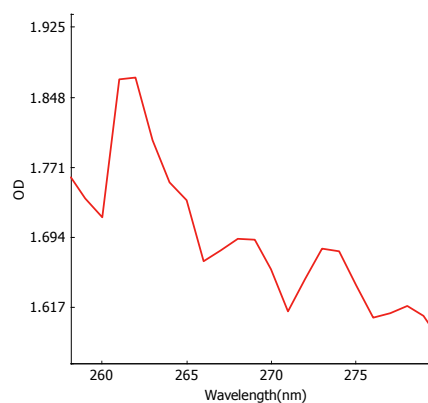


Figure 24: - UV-Visible spectrum of reduced graphene oxide prepared using *Eichhornia crassipes* aqueous extract

The UV–Vis spectrum shows an absorption peak at 234 nm for graphene oxide corresponding to the $p-p^*$ transition of aromatic C–C bonds. The absorption peak for graphene at 274 nm confirms the reduction of graphene (**Firdhouse et al., 2013**).

A red shift UV for reduced graphene oxide which is due to the electronic configuration in graphene in the reduction of graphene oxide. The absorption peak of graphene oxide attributed to $\pi-\pi^*$ transition of aromatic C–C ring. The UV spectra of

reduced graphene oxide on the other hand show the red shift. This absorption peak is attributed to $n-\pi^*$ transition of C–O bonds now embedded by exfoliation and intercalation on the graphene (Thema *et al.*, 2013).

4.4 UV spectrum for nano composite

UV spectrum taken for the composition of gold nano particles and reduced graphene oxide using *Eichhornia crassipes* is given in figure 25.

No. 56
 Sample Name : COMPOSITE
 Measurement Mode : Photometric
 Wavelength(nm) : 220 250 320 370 430 500 600 780
 DateTime : 17/04/04 13:00:22

OD220 : 6.652
 OD250 : 4.507
 OD320 : 2.209
 OD370 : 1.291
 OD430 : 0.777
 OD500 : 0.466
 OD600 : 0.249
 OD780 : -0.467

Item	Result
Pathlength (mm)	0.189
Dilution	1.000

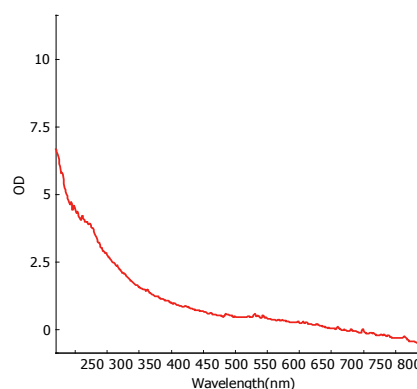


Figure 25. UV-Visible spectrum for nano composite

4.5. Characterization of nano compounds

4.5.1 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR is a rapid, nondestructive, time saving method that can detect a range of functional groups and is sensitive to changes in molecular structure. The synthesized gold nano particles and reduced graphene oxide using *Eichhornia crassipes* plant extract were analysed in FTIR spectrum.

In FTIR spectra the finger print region of carbonyl groups shows C=O stretching in the region 1650-1950 cm^{-2} . Absence of carbonyl peak in the FTIR of graphene indicates the formation of reduced graphene oxide in **(Fig. 26)**.

The FTIR spectra of AuNPs with absorption peaks at 3350.22, 3842.20, 3749.62, 3448.72, 2924.09, 2376.30, and 601 cm^{-1} were observed **(Fig. 27)**. The spectra obtained to characterize the interaction between HAuCl_4 and plant extract has a strong peak at 3448.72 cm^{-1} showing the presence of -OH group (stretch H bonded, strong broad) and 2924.09 cm^{-1} shows -C-H stretching along with the above mentioned peaks, δ C=O is observed at 601.79 cm^{-1} . In IR, peaks 1627 cm^{-1} may be due to stretching vibrations of -C=C. All the above the peaks indicate the functional groups in the plant extract embedded gold nano particles and the metabolites present in gold nano particles responsible for the reducing property of the extract.

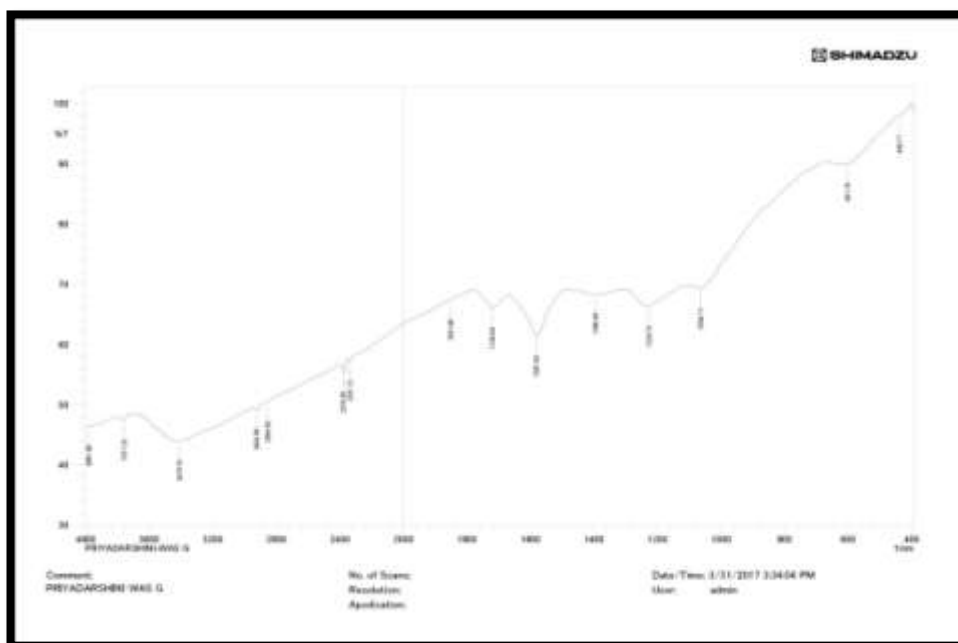


Figure 26. FTIR spectrum of reduced graphene oxide

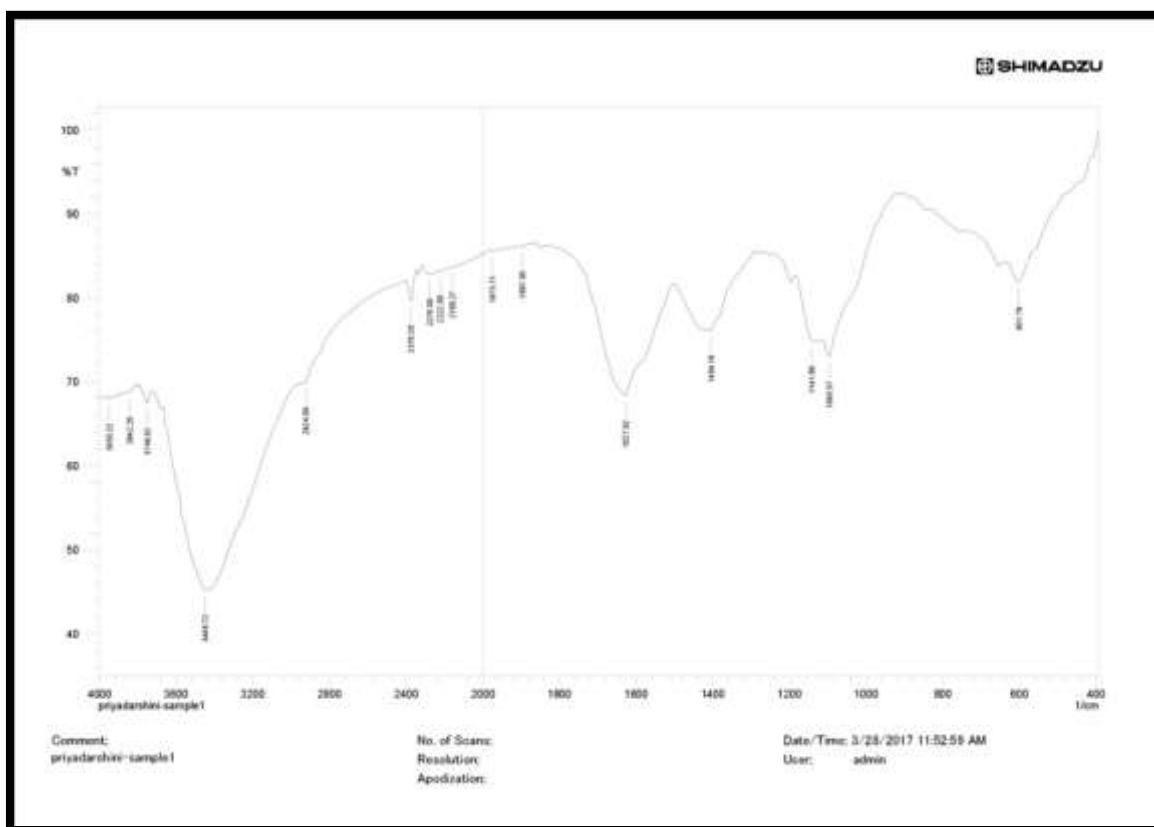


Figure 27. FTIR spectrum of gold nano particles prepared by Hot Air Oven method

4.5.2 Scanning Electron Microscopy (SEM) analysis of synthesized nanoparticles

The surface morphology of graphene oxide investigated by means of Scanning Electron Microscopy (SEM) with the EDX detector is given in **figure 28**.

Its shows highly-wrinkled graphitic layers proving that there is a distortion in the graphene layers due to the linkage of the residual oxygen after thermal reduction, while large nanosheet sizes are preserved.

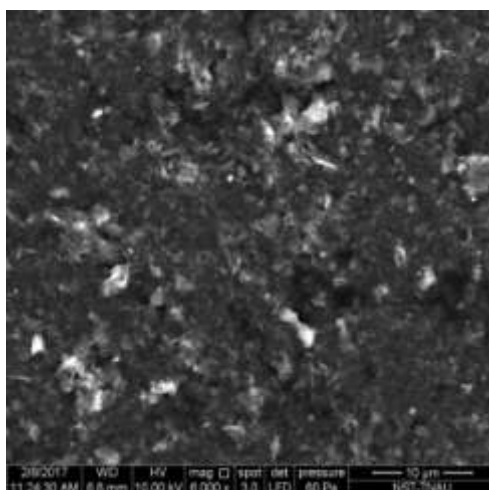


Figure 28. Surface morphology of the reduced graphene oxide

4.6 Anti-proliferative efficiency of the synthesized nanoparticles using MTT assay

The results of the cell growth inhibition by gold nano particles, reduced graphene oxide and its composite against MCF-7 breast cancer lines for various concentrations 50µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml, 250 µg/ml is given below. Doxorubicin Standard used for this analysis and IC₅₀ – Values of respective Compounds taken at 48 hrs.

As the concentration of the gold nano particles increases the cell growth decrease and it was found to be the higher anticancer activity of nano particles at 200µg/mL **Figure 29.**

The results obtained in the present study showed that reduced graphene oxide using *E. crassipes* had a very moderate anticancer activity at 50 µg/ml. Increase in the concentration of the samle decreases the percentage of cell viability. **Figure 30** Shows that the reduced graphene oxide has anticancer property against MCF-7 cell lines.

Figure 31 shows the anticancer property of the composite made of gold nano particles and reduced graphene oxide. With increase in concentration the cell viability decreases as given in **table 7.**

Table 5. Percentage of Cell viability of gold nano particles against MCF-7 cell lines

Concentration of gold nano particles(µg/mL)	% Cell viability
50	96
100	53
150	42
200	35
250	35

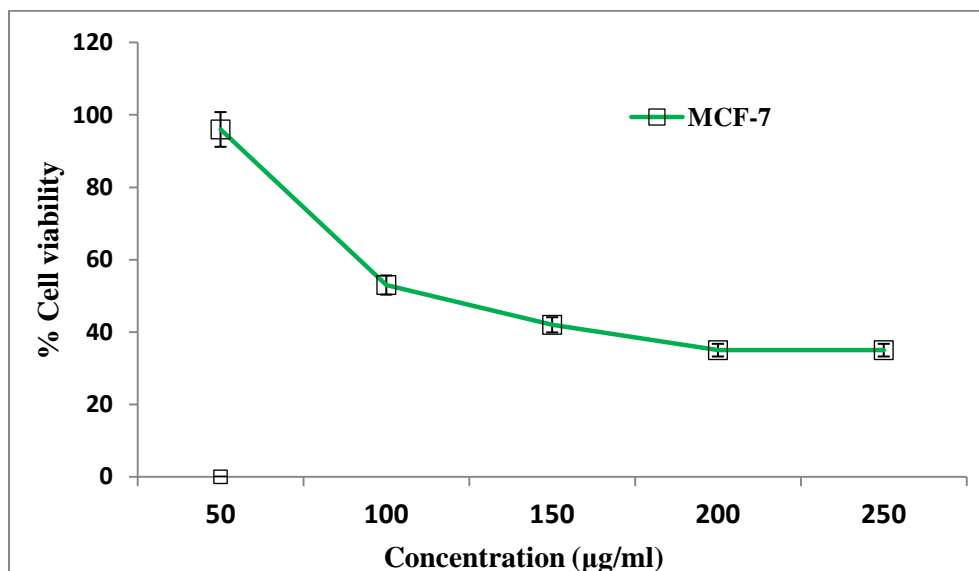


Figure 29: Anticancer activity of synthesized gold nano particles against MCF-7 cell lines

Table 6. Percentage of Cell viability of few layer graphene against MCF-7 cell lines

Concentration of gold nano particles(µg/mL)	% Cell viability
50	97
100	75
150	54
200	29
250	23

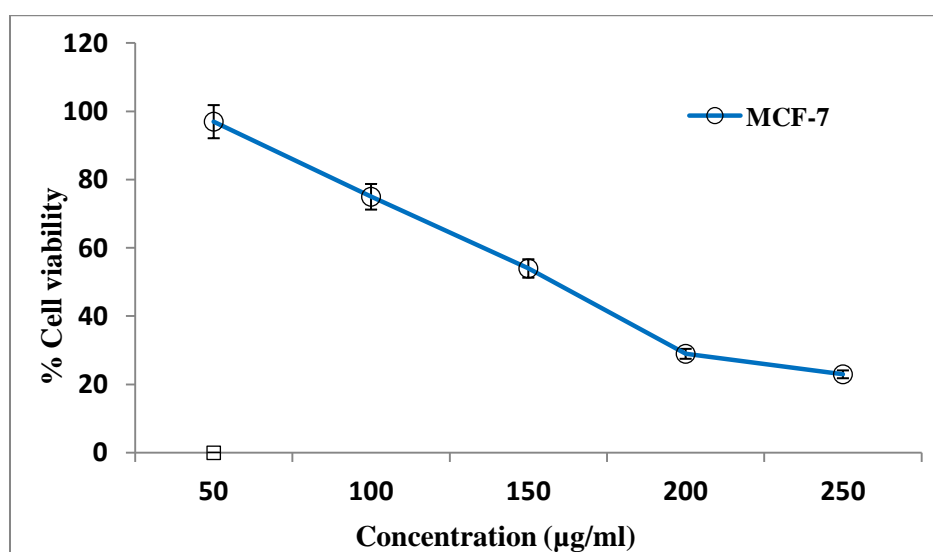


Figure 30: Anticancer activity of synthesized reduced graphene oxide against MCF-7 cell lines

Table 7. Percentage of Cell viability of composite against MCF-7 cell lines

Concentration of gold nano particles($\mu\text{g/mL}$)	% Cell viability
50	96
100	65
150	56
200	44
250	40

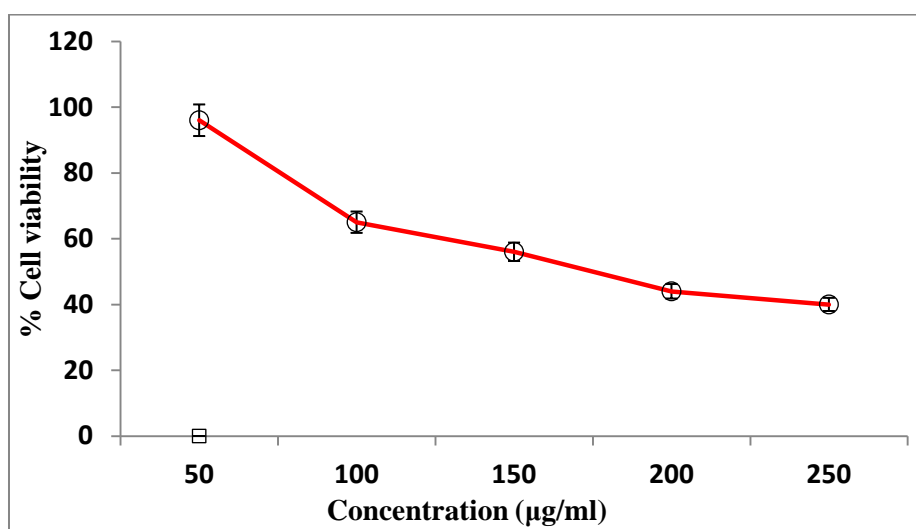


Figure 31: Anticancer activity of composite of WHRGO and WHAuNP'S against MCF-7 cell lines

The present study shows the cytotoxic activity of the compounds viz. reduced graphene oxide, gold nano particles and nanocomposite against MCF-7 cell line as given in the **table 8**.

Table 8. Cytotoxic activity of Complexes ($\mu\text{g/ml}$)

Compound name	MCF-7 Cytotoxicity %
CS	120 \pm 1.0
RS	140 \pm 0
GS	120 \pm 1.0
DOX	16 \pm 1.5

CS-Composite made out of nanogold and nanographene; RS-Reduced graphene oxide; GS- Gold nano particles; DOX-Standard.

These results clearly portray that the compared to standard the samples used in the study give appreciable results. The present research work substantiates the use of nanoparticles and also water hyacinth in anti-cancer studies.

4.7 Solar cell application of synthesized nano compounds

Synthesized gold nano particles (8 μl -10 μl) , reduced graphene oxide (8 μl -10 μl) and its composite (8 μl -10 μl) , plant extract of water hyacinth (10 μl -12 μl) in different combinations were separately coated onto FTO plates for recording the use of these nanomaterials in Dye Sensitizing Solar Cell applications. Photovoltaic measurements employ a 40W tungsten lamp. The light absorbance of the prepared solar cell was confirmed with measure the current and voltage of the solar cell and current and voltage characteristics of the synthesized nano compounds at different time interval is given in **table 9**. IV graph was plotted for the prepared solar cell and I_{sc} : 6.43 Micro Amp, V_{oc} : 0.496 Volt values are obtained.

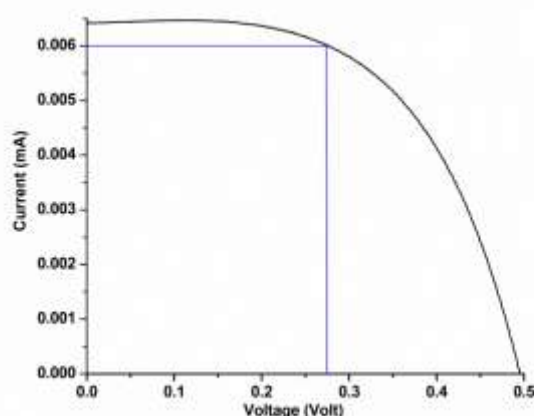


Figure 32. IV Graph of prepared solar cell using water hyacinth plant extract

Table 9. Current and voltage characteristics of solar cells fabricated with prepared nano particles

Size of the plate	Sensitizer	Current (μA)			Voltage(V)		
		Zero timing	After 5 exposing	After 10 exposing	Zero timing	After 5 exposing	After 10 exposing
1.3x1.3	WHRGO	0.71	3.11	00.26	0.1779	0.098	0.0056
1.3x1.3	WHAuNP'S	0.015	02.65	1.26	0.161	0.3447	0.0134
1.3x1.3	Composite	0.03	01.25	00.24	0.004	0.0543	0.0201
1.3x1.3	WH extract	0.05	0.4572	0.39	00.38	0.538	0.3744

The light-scattering layer has been shown to act as a photon-trapping system and equally active in photovoltaic generation itself (**Zhang et al., 2007**). Cell efficiencies measured were 5% in dye sensitized solar cells using only the light-scattering TiO₂ layer. The short circuit current density and cell performance significantly increase as nanorods length increases because a higher amount of the adsorbed dye on longer nanorods, resulting in improving conversion efficiency (**Kim et al.,2006**). Electrolyte containing I⁻/I₃⁻-redox ions are used in DSSC to regenerate the oxidized dye molecules and hence completing the electric circuit by mediating electrons between the nanostructured electrode and counter electrode (**Seigo Ito et al .,2008**).

The present study reveals that the water hyacinth mediated graphene obtained by reducing graphene oxide when used as coating material in the fabrication of solar cell gives appreciable results revealing the scope and viability of use of water hyacinth in the synthesis of nanomaterials and thence its use in solar cells

SUMMARY AND
CONCLUSION

5. SUMMARY AND CONCLUSION

Based on the Results and Discussions the study on “**Bio fabrication of gold and graphene nano particles –Anticancer and Solar cell application**”, the following are the conclusions arrived at:

- 🌍 Preliminary Phytochemical test of the extracts of *E. crassipes* (Mart.) Solms. showed the presence of various metabolites such as alkaloids, flavonoids, sterols, anthroquinone, quinones and carbohydrates.
- 🌍 From the literature the metabolites present in the *E. crassipes* (Mart.) Solms extracts which might have been responsible for the synthesis of gold nano particles.
- 🌍 Various methods were employed to synthesize gold nano particles by the reduction of gold chloride with plant extract.
- 🌍 In room temperature method the gold nano particles were produced in 8 hours.
- 🌍 In sonication method gold nano particles using plant extract were formed within 10 minutes.
- 🌍 In microwave assessed method the gold nano particles were produced in 45 seconds.
- 🌍 The gold nano particles were synthesized using oven heating method with different concentrations of the plant extract and the constant amount of gold chloride viz versa. The gold nano particles formed within 5 minutes.
- 🌍 In oven heating method the concentration the plant extract increases the time taken to form the gold nano particles al so increases, similarly gold chloride solution concentration increases the formation of the gold nano particles were increased.
- 🌍 Reduced graphene oxide was prepared using *Eichhornia crassipes* by Hummer’s method.
- 🌍 The gold nano particles and Reduced graphene oxide were characterized using Surface Plasmon Resonance was obtained in the range of 520 – 540 nm and 262 nm respectively.
- 🌍 FTIR analysis shows the formation of gold nano particles and reduced graphene oxide. Selected groups in the FTIR of plant extract were found to be missing in the nanoparticle embedded plant extract revealing the role of these functional groups in the reduction process
- 🌍 The SEM analysis shows the the surface of the synthesized reduced graphene oxide. Reduced graphene oxide shows highly-wrinkled graphitic layers.
- 🌍 Bio synthesized gold nano particles and reduced graphene oxide were tested for cell viability for 50 µl, 100 µl, 150µl, 200 µl, 250 µl respectively. The anti cancer activity of the synthesized compounds against MCF-7 cell line were confirmed.

- 🌍 Prepared nano compounds were used to fabricate environment- friendly solar cells.
- 🌍 The synthesized nanomaterials on a 1.3x1.3cm plate are capable to produce voltage and current with micro amounts of compound.
- 🌍 Increasing the amount of compound used as coating materials and increasing the size of the plate current and voltage, and concentration of the electrolyte were found to be some of the dependant factors in solar cell fabrication.
- 🌍 Use of different lamps also affects the photovoltaic performance. A 40V tungsten lamp produces more current then room temperature.

Scope for future work:

- ✓ *Anticancer activity study of reduced graphene oxide obtained using Eichhornia crassipes with other cancer cell lines*
- ✓ *Preparing and characterization of the single layer graphene oxide*
- ✓ *Improving the current voltage for calculating the fill factor and efficiency is needed and the application of these solar cells is recommended as future work.*

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