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Monowave synthesis of Palladium nanoparticles by Sterculia Urens gum (karaya) activity on MCF-7 Human Breast Cancer Cells and antioxidant activity

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ABSTRACT

A Monowave synthetic route for the production of Palladium nanoparticles using Gum karaya(GK) is examined for in vitro cytotoxic efficacy against MCF-7. The present work proves the influence of gum karaya composition on the size and morphology of the produced Palladium nanoparticles. The biosynthetic method is quick, simple and easily synthesized and color changes observed in Ultraviolet and visible spectroscopy. Subsequently, X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR) and Transmission electron microscopy (TEM) analyses were employed to characterize the as synthesized GKPdNP. The XRD investigation confirmed the presence of Pd (0) nanoparticles and interactions between the bio degradable compounds of the Gum and the produced GKPdNP were confirmed by the FTIR spectra. TEM images demonstrated that the nanoparticles has a size distribution of 3 ± 2 nm. Moreover, the anticancer activity of the GKPdNP was tested against MCF-7 strains. All synthesized Pd nanoparticles exhibited good antioxidant activity.

INTRODUCTION

The improvement of Nanotechnology research is a current interesting and investigation field that contains plan, synthesis and utilization of particles ranging in dimensions from around one to hundred nanometers [1, 2]. Present day's palladium, platinum, gold and silver metal nanoparticles have great applications in homogeneous and heterogeneous catalysis. Noble metal nanoparticles have been widely studied for many years because they usually show unique properties and can be potentially used in various areas, including applications in therapeutics, catalysis, optics, biodiagnostics [3], medical and electronic fields. Excellent catalytic activity of Palladium in various industrial processes made the Pd nanoparticles to be used as catalysts in

many coupling reactions such as, Suzuki coupling [4], Heck coupling [5] and hydrogenation of allyl alcohols [6]. Recently, Noble metals like Palladium, Silver, Platinum, Gold nanoparticles have grown much interest among the developing the therapeutic potential in treating a variety of diseases acting as bactericidal, antioxidant and anti-inflammatory agents. They are also used in the areas like imaging, drug delivery, cancer therapy, cell labeling.

Most common cause of cancer death in women is Breast cancer [7, 8] and it originates from the tissue of breast, mainly from the inner lining of lobules or milk ducts. The growth of breast cancer includes numerous proteins and genes, namely; CDK 2 (Cyclin dependent kinase), VEGF (Vascular endothelial growth factor), HIF 1 (Hypoxia-inducible factors), AP 1 (Activator protein), p53, p57, cyclin A and B 7 [9]. At this time available chemopreventives and chemotherapeutic agents cause adverse side effects, therefore, increasing a biocompatible and cost effective process of cure to cancer treatment is essential. Current research in the nano-oncology takes headed up the progress of many nanoscale materials, devices and healing agents for the early diagnosis and treatment of cancer [10].

However, synthesis of PdNPs involved various chemicals such as PEG [11], PVP [12], PVA [13], NaBH₄ [14]. These chemicals result in harm environment and have impact on greenhouse outcome. Over the last few years there has been an improved interest in the green synthesis, and the nanoparticles synthesis using plant extracts would be economical due to easier and friendly processing [15]. Latest outcomes showed that palladium nanoparticles were prepared in greater numbers using coffee and tea extracts at room temperature without using surfactants, capping agents and templates [16]. Many research groups have been reported the palladium synthesis using various secondary metabolisms which are present in the plants of *Curcuma longa* tuber [17], *Cinnamon zeylanicum* bark [18], Gum acacia [19], Xanthan gum [20].

In this paper, for the first time, we intend to prepare the nano-crystalline palladium using Gum karaya (GK) as the reducing and stabilizing media and exploit its potential use. GK is also termed as *sterculia* gum, is the dried exudation negative colloid and a high-molecular-weight complex acidic polysaccharide. The primary structure is shown to be composed of D-galactose, D-glucuronic acid, D-galacturonic acid, L-rhamnose and acetyl groups, in different proportions according to the quality, type, and origin of the polysaccharide. The gum has a number of

applications in pharmaceutical, leather, bakery and dairy industries [21]. In this paper, we report the green synthesis of PdNPs using GK (GKPdNP) as both reducing and stabilizing agent without using any hazardous chemicals. The exclusivity of this reported technique is the high rate of synthesis using Monowave 50 at 150 C° up to 20 mins. Therefore, this study was taken up to produce GKPdNP to evaluate potential toxicity and the overall mechanism of treating the human breast cancer cells (MCF-7 cells). Furthermore, the green synthesized GKPdNP catalytic activity towards the reduction of ferric to ferrate has been investigated by UV-visible spectrophotometric method. An attempt has been made to examine the antioxidant activity of GKPdNP by using widely known free radical scavenging model system i.e., 2,2-Diphenyl-1-picrylhydrazyl (DPPH) by the recordings on UV-visible spectroscopy.

MATERIALS AND METHODS

SYNTHESIS OF GKPdNP

The GK grade 1, was bought from Girijan co-operative corporation, Visakhapatnam, A.P., was cleaned, washed thoroughly with double distilled (DD) water and dissolved 1g of GK in 100 ml of DD water. An aliquot of 50 ml of the aqueous H_2PdCl_4 ($5 \times 10^{-4}\text{M}$) solution was mixed with 50 ml of 1% aqueous solution of GK in a beaker. The reaction was carried out in a Monowave at 150 C° for (15, 18, 21, 24) min.

CELL CULTURE: Breast cancer cells of Human (MCF-7) were received from NCCS, Pune. The cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 1% antibiotic-antimycotic solution and 10% fetal bovine serum (FBS) was bought from chemicals of Hi-media. These cells were grown-up to confluence together at 37 C° with 5% CO₂ media. Entire studies were performed in 96-well plates. MCF-7 cells were seeded onto the plates at a density of 1×10^2 cells per well and incubated for 24 h prior to the tryouts. 1 mg of synthesized GKPdNP were first liquefied in 10 ml of double distilled water. Experiments were done using varying concentrations of GKPdNP (6.25, 12.5, 25, 50 and 100 µg/mL) and compared with GK (6.25, 12.5, 25, 50 and 100 mg/mL) in variable concentrations and control.

At the completion of 24 h the medium in every well was thrown away and 10 µL of MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) solution (5 mg/mL in phosphate buffer saline) were mixed with every well and all the plates were incubated at 37 °C.

On completion of 4 h, DMSO (100 μ L) was added to every well to dissolve formed formazan crystals and absorbance was recorded at 597 nm filter using ELISA plate reader (Lisa plus, India) to estimate percent cell viability.

$$\% \text{ Viability} = (\text{AD} - \text{AA}) / (\text{AF} - \text{AA}) \times 100$$

Where, AD = Absorbance of experimental cells (drug), AA = Absorbance of blank (only media), AF = Absorbance of control (untreated)

So,

$$\% \text{ Cytotoxicity} = 100 - \% \text{ cell existence}$$

The cytotoxicity test was monitored by the technique reported in literature [22]. The cells were grown at 1×10^4 cells / well into a six well chamber plate and later incubated overnight. Further, the medium was substituted with followed medium DMEM without FBS having (6.25, 12.50, 25 50 and 100 μ g/ml) GKPdNP and GK at 24h in vitro cell line. The cell structure was inspected under Nikon inverted microscope.

ANTIOXIDANT ACTIVITY

The antioxidant activity of GKPdNP was studied using stable DPPH radical, which was working for the study of radical scavenging property of GK following by Brand Williams method [23]. A ethanol solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) i.e., 1 mg in 100 ml ethanol was prepared and 3.0 ml of this solution was mixed with various concentrations (15, 30, 45, 60 & 75 μ g/mL) of synthesized GKPdNP, GK and control Ascorbic acid (AA). The reaction mix was shaken thoroughly and incubated at room temperature for 30 min. The color change from violet to color less and absorbance intensity at 517 nm was studied by UV–visible spectrophotometry. DPPH inhibition (%) was evaluated by the following equation

$$\text{DPPH \% inhibition} = [(\text{Abs control} - \text{Abs sample}) / (\text{Abs control})] \times 100$$

Abs control is the absorbance of DPPH radical + methanol; Abs sample is the absorbance of DPPH radical + synthesized GKPdNP solution. Concentration of GKPdNP, GK and AA vs inhibition % of DPPH was obtained.

Results AND Discussion

UV-Visible Spectroscopy analysis

The H_2PdCl_4 and GK yellow color solution, subjected to UV-Visible spectroscopic study, has shown peak at 417 nm and after formation of GKPdNP, the solution turned to black color and broadening of the peak was observed as shown in Figure 1, at various time (15,18,21,24 min) intervals. The time dependent absorbance peak indicated that the GKPdNP were synthesized in bulk amount and synthesis was highly effective, without the addition of any toxic chemicals.

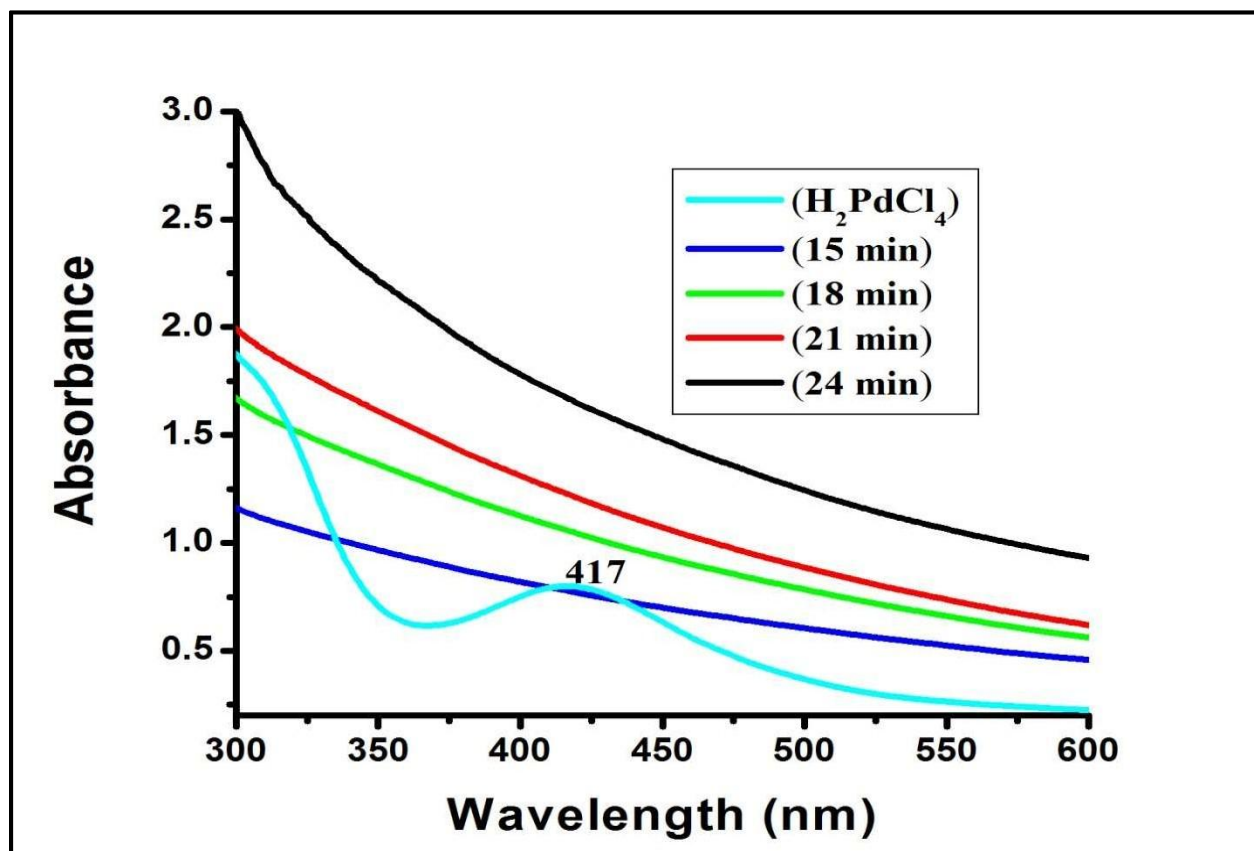


Figure: 1 UV-visible analysis of GKPdNP

FTIR analysis

The interaction of hydroxyl groups of GK in the stabilization and reduction of the GKPdNP was found from the FTIR spectra as shown in Fig. 2. FTIR spectrum of the GK absorption bands at 3438 cm^{-1} represents the -OH stretching and the band at 1620 cm^{-1} is due to asymmetric and symmetric stretching of COO^- group. However, 2934 cm^{-1} band represents -CH_2 , -CH_3 aliphatic groups and the band at 1427 cm^{-1} is due to -OH bending of acid group. The changes in the shape and peak positions of -COO^- , -OH , groups as 1620 and 3447 cm^{-1} respectively. Thus, the green, cheap and naturally obtained GK has worked as a reducing agent and also stabilizing agent.

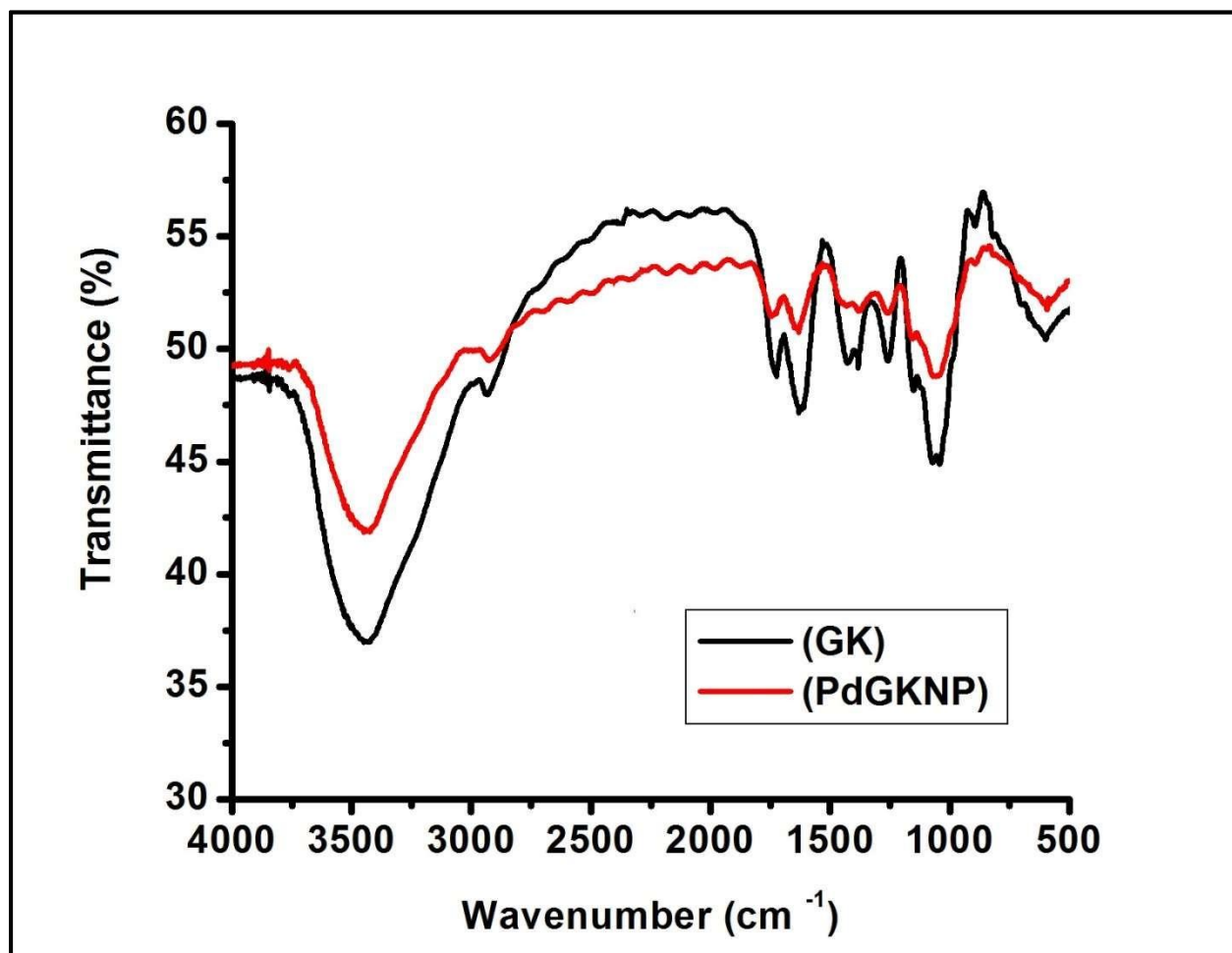


Figure: 2 FTIR analysis of GKPdNP

XRD Analysis

The peaks at the 2θ values 39.06, 46.23, 67.56 and 78.1 representing the miller indices planes at 111, 200, 220 and 311 are the crystallographic planes of face centered cubic (FCC) structure of palladium crystals respectively. These planes confirmed the GKPdNP synthesized by using GK was crystalline in nature Fig. 3. The sizes of the GKPdNP are calculated with the (111) reflection by the formula of Scherer with a geometric factor of 0.97.

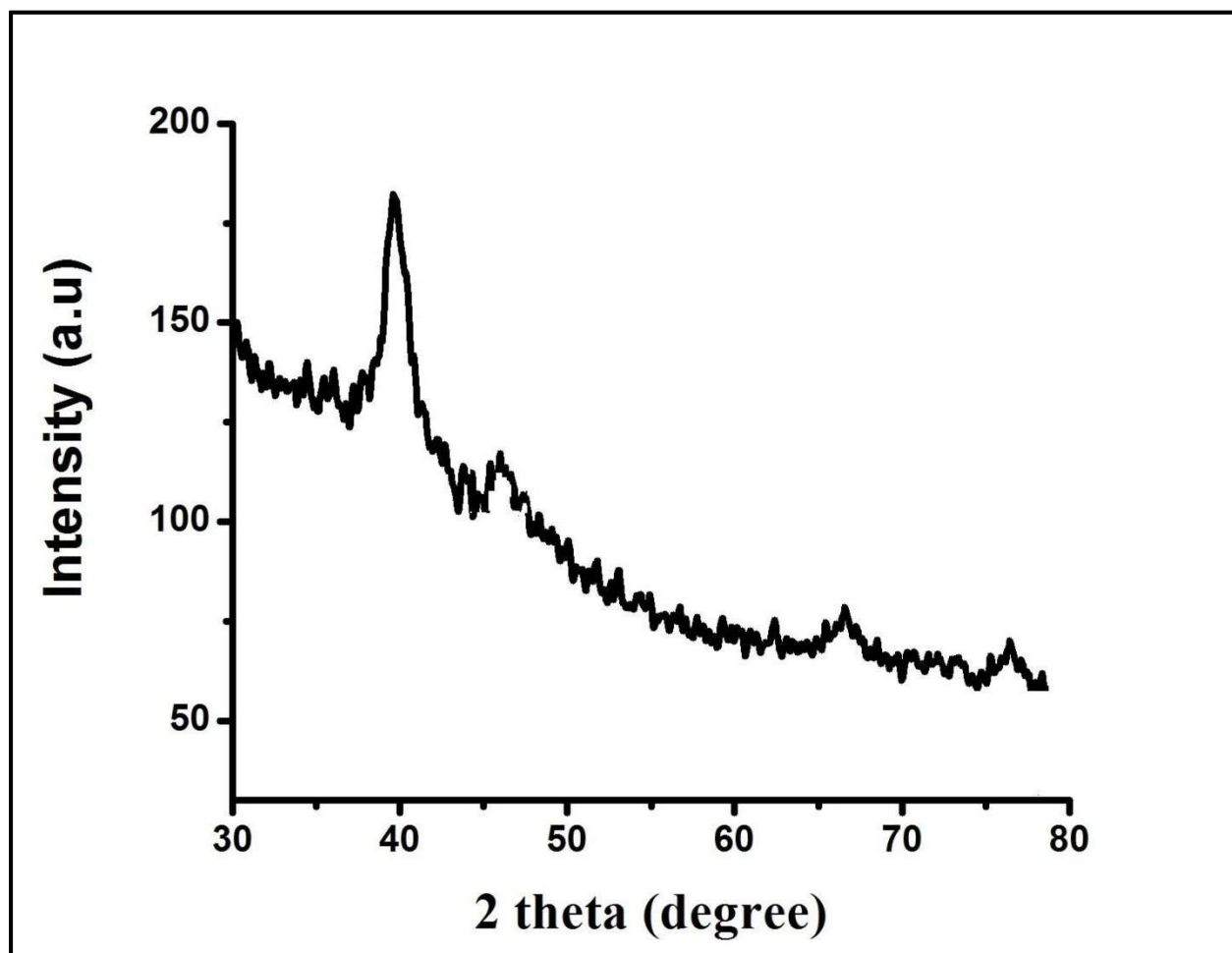


Figure: 3 XRD analysis of GKPdNP

TEM analysis

TEM images of the synthesized GKPdNP with GK acting as a reducing and stabilizing agent, are shown in Figure 4 (a). The TEM analysis is an easy way to find out the size and morphology of the nanoparticles. It confirmed the nano particles to be spherical in shape, well dispersed and not

in physical contact, which were evenly distributed by biomolecules. Histogram in the Figure 4 (b), showed that average diameter of the particles was 3 ± 2 nm.

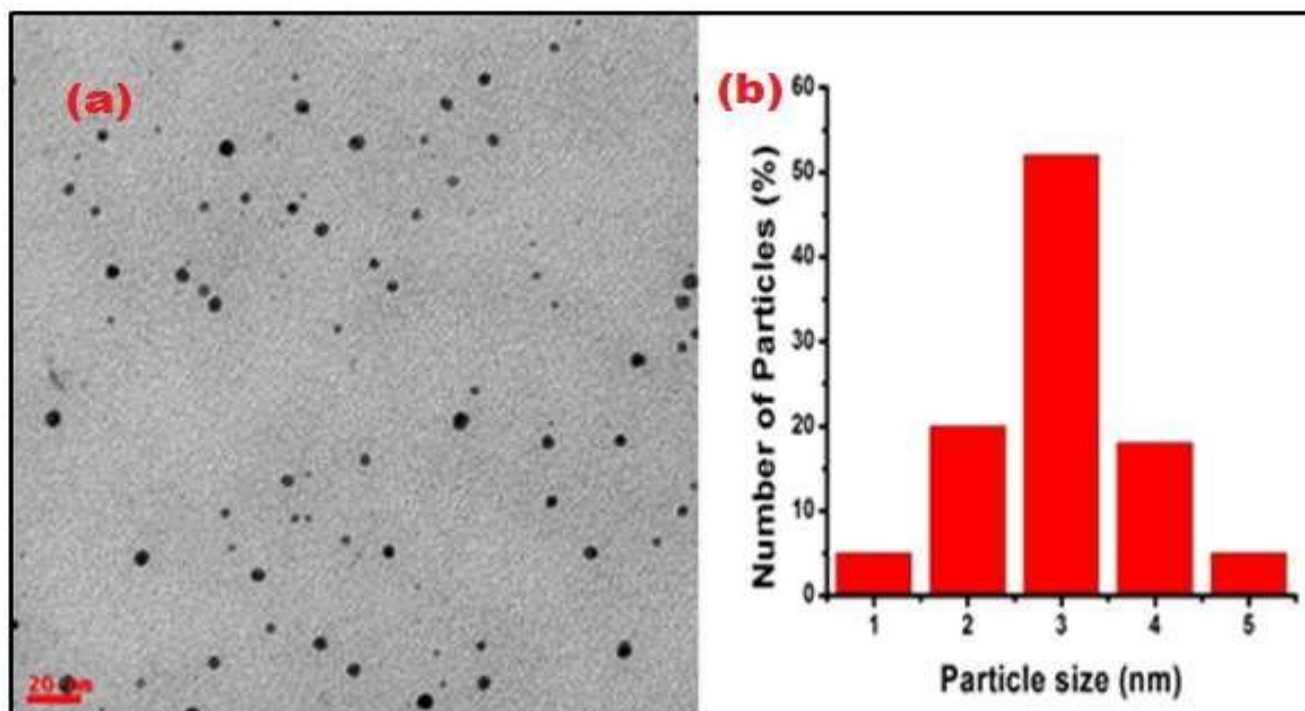


Figure: 4 (a)TEM analysis of GKPdNP (b) Histogram

In vitro anticancer activity of GKPdNP against MCF-7 cell lines

Cell viability of synthesized GKPdNP nanoparticles was examined by in vitro MTT assay on MCF-7. The performance of biosynthesized GKPdNP on breast cancer cell lines has been studied [22]. There are few reports on the study of MTT assay on MCF-7 breast cell lines, which have reported anticancer activity of PdNPs [24]. In this paper we reported novel green synthesis of GKPdNP at various concentrations (12.5, 25, 50 and 100 $\mu\text{g/mL}$) and applying to MCF-7 cells for 24 hours and confirming % cell development inhibition by using MTT assay. Cell viability was measured using equation 1. The cell viability decreased when dosage concentration increased (6.25, 12.5, 25, 50 and 100 $\mu\text{g/mL}$). As a result, significant reduction in cell viability was observed when treated the MCF-7 cells with GKPdNP at 100 $\mu\text{g/mL}$ concentration. Treated cells were shrink, spherical, attached and aggregated when compared to GK. The viability of tumor cells was confirmed by using neutral red assay. The results revealed that at a concentration of IC_{50} 46.79 $\mu\text{g/mL}$, tumor cells death was due to the biosynthesized GKPdNP. The figure (5)

has shown that the synthesized GKPdNP treated cells because variation in cell viability as the concentration is increased. This study concludes strongly that the biosynthesized GKPdNP possess excellent anticancer activity against MCF-7 cells.

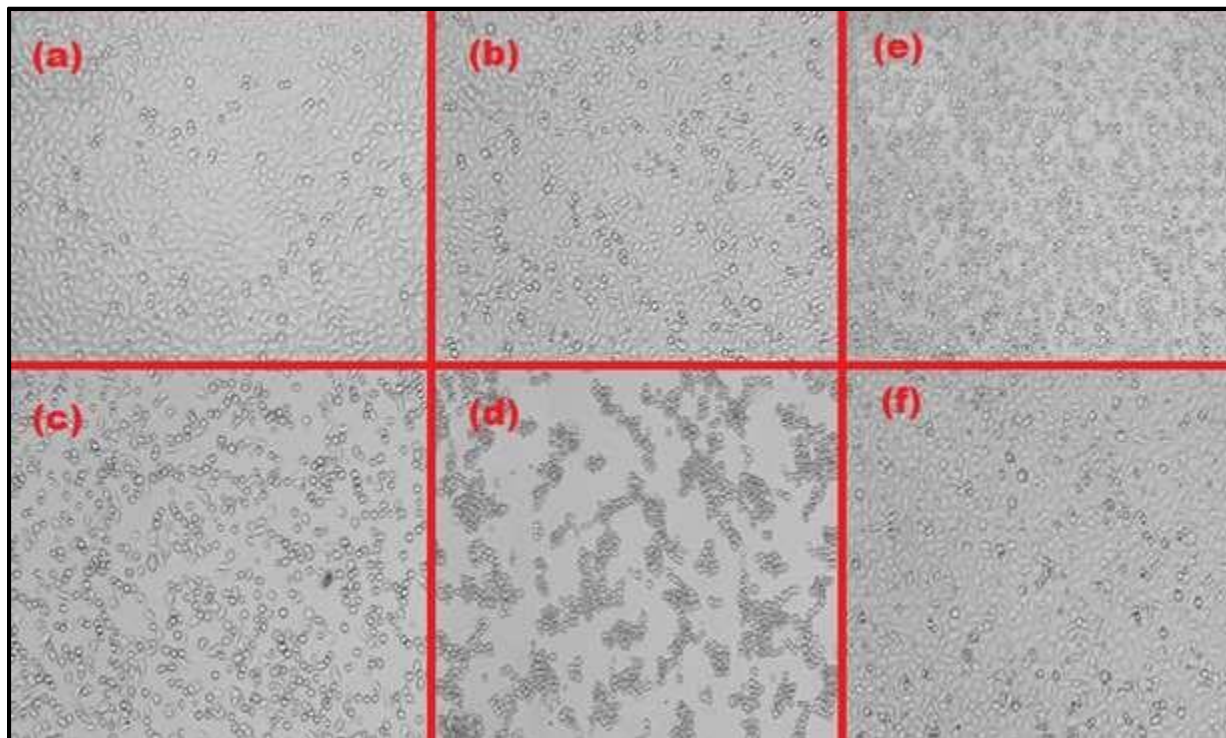


Figure:5 (a) MCF-7 control untreated cells (b) 6.25 µg/mL (c) 50 µg/mL (d) 100 µg/mL (e) DOX (f) GK

Antioxidant activity of GKPdNP

In vitro antioxidant activity of the synthesized GKPdNP was investigated by taking five antioxidant measurements which revealed the antioxidant potential of the GKPdNP. Figure 7 shows the dose response for the DPPH scavenging activity at varying concentrations of the GKPdNP (15, 30, 45, 60, and 75 µg/mL) and observance of significant scavenging activity of GKPdNP at IC₅₀ 51.11 µg/mL). However, these activities are not more than that of control ascorbic acid. The reductive capabilities of the biosynthesized GKPdNP are shown in Figure 6. The reducing power of the GKPdNP has increased with increase in the amount of sample.

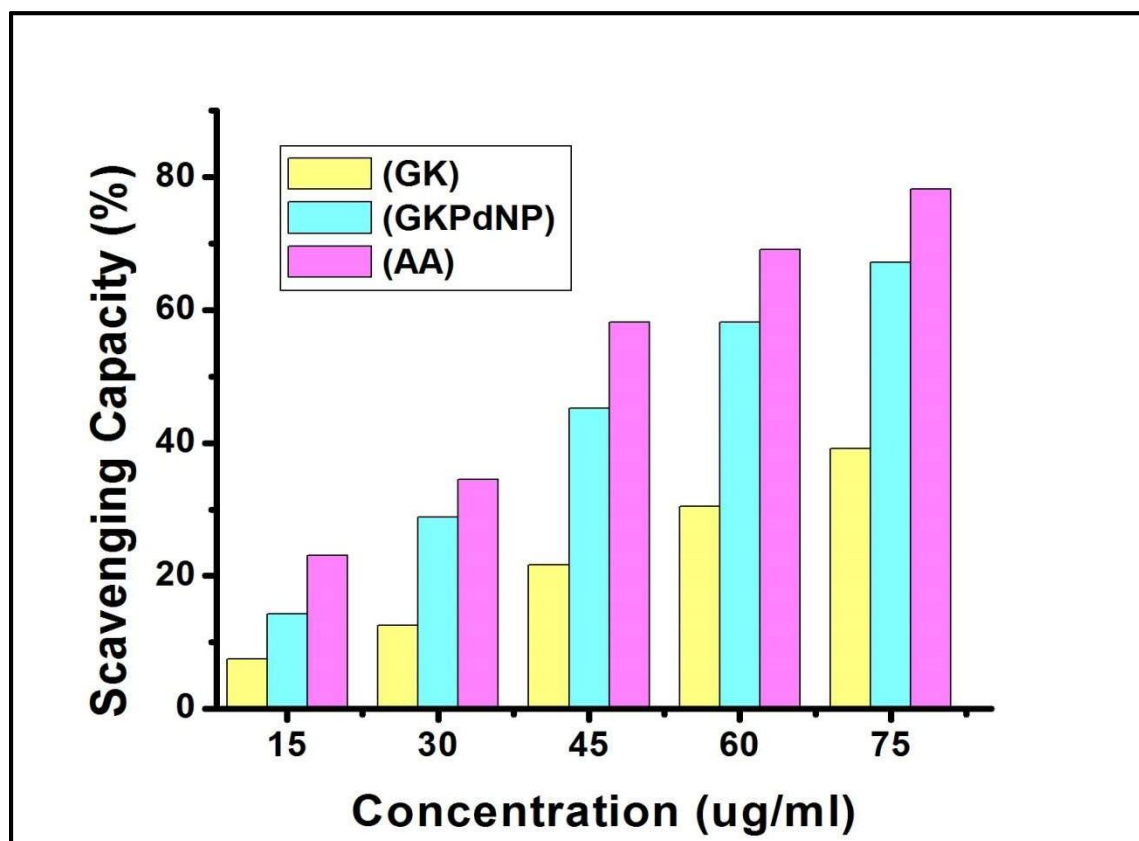


Figure: 6 Antioxidant activity of GKPdNP

Conclusion

PdNPs were effectively synthesized using the GK with aqueous H_2PdCl_4 solution. This method is fast as well as convenient. The accountability of biomaterial, responsible for the reduction of metal ions (Pd^{+2}) was studied using UV-visible spectroscopic analysis. Crystalline nature and face-centered cubic structure of GKPdNP with an average particle size around 3 ± 2 nm were confirmed by XRD analysis and compared with standard elemental configuration. The morphology studies of the biosynthesized GKPdNP were carried out using TEM analysis and further characterization was done using FTIR analysis. GKPdNP showed potent anti-proliferative efficacy against breast cancer MCF-7 cells with IC_{50} value of $46.79 \mu g/mL$. The free radical scavenging activity of both biologically synthesized. Examination of GKPdNP and GK revealed that scavenging ability % has increased with increasing the concentration and at IC_{50} $51.11 \mu g/mL$. This investigation describes the cost-effective and eco-friendly biological method for the synthesis of GKPdNP for effective anticancer and antioxidant activities.

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