# Microwave-assisted synchronous nanogold synthesis reinforced by kenaf seed and decoding their biocompatibility and anticancer activity

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## Introduction

At present, cancer has been one of the most significant causes of human death around the globe. Over the past two decades, numerous developments and progressions in various cancer treatment approaches (e.g., surgery, radiation, and chemotherapy) have been recognized in hospitals. A great deal has been undertaken to improve these conventional patterns, which already have a range of limitations, including poor efficiency, extreme adverse reactions, and a greater cancer recurrence. Therefore, interventions with fewer adverse effects and better clinical performance need to be developed. Nanomedicine's present advancement has made it possible for us to produce many innovative nanomaterials for parallel assessment and treatment.

Current nanotech research uses a vast range of emerging nanomaterials in cancer therapy. Gold nanoparticles (GNPs) are mainly used for various diseases, with multiple benefits, as nanomedicine. They can safely be used in systemic circulation due to their stability and size variance, which is why researchers have concentrated on GNPs for future cancer therapy applications. GNPs can be generally produced with a number of shapes (sphere, rod, branched structure, cage-like, etc.), and it can range in size from 1 nm to 100 nm or more. Because of the surface charge, GNPs are comfortably functioned by different types of biomolecules (i.e., drugs, genes, and targeting ligands). Hence, GNPs are the most responsive material for different biomedical applications considering all these unique features.

An industrially valuable plant, “Kenaf (*Hibiscus cannabinus*)” possesses remarkable anti-cancer, anti-inflammatory, anti-obesity, and antioxidant medicinal properties. Mainly, kenaf seed is the potential source of various health-promoting compounds, such as phenylpropanoid compounds, sterols, kaempferol, omega-3-fatty acids. Hence, it has implausible demand in both the food and pharmaceutical industries. Previously, kenaf seed and kenaf seed-based silver nanoparticles exposed promising anti-lung cancer and antibacterial activities. Besides, biopolymer mediated nanocomposites were prepared using kenaf seed in order to enhance its antioxidant capacity. However, abundant bioactive compounds and outstanding biological attributes make the kenaf seed a candidate for further research.

In this contribution, we have predominantly focused on the rapid synthesis of kenaf seed capped gold nanoparticles (KS@GNPs) due to their groundbreaking anti-cancer properties. Here, kenaf seed was used as both a reducing and capping agent for the synthesis of GNPs, followed by employing MWI as an easy-to-operate technique.

## Materials and methods

**Kenaf seed-mediated gold nanoparticles synthesis (KS@GNPs)**

The prepared KS extract was used to synthesize gold nanoparticles (KS@GNPs) by reducing and capping of HAuCl4 under microwave irradiation (MWI). To state the process, KS (1 g) was added to Milli-Q water (100 mL) and sonicated (1 h) and stirred (4 h) to get well-mixed stock solution (1 %). Afterwards, stock solution (30 mL) was mixed with HAuCl4 (10 mL) in a glass vial (20 mL) and subjected to MW (Midea, MC-E230KW, and 800 W) until the reaction mixture turned into a blushing red color. The final concentration of KS and HAuCl4 were 1 % and 1 mM, respectively, and MWI time was 90 s. However, the overall synthesis conditions were optimized systemically with the concentration of KS (0.1 to 1 %), HAuCl4 (0.1 to 1 mM), and MWI time (30 to 90 s) by changing one parameter while keeping other parameters constant. The final biosynthesized KS@GNPs were centrifuged (10 min at 14000 rpm) and obtained pellets (3 times washed) were freeze dried and preserved for further characterizations.

**Characterizations of KS@GNPs**

The reaction conditions of KS@GNPs were investigated by UV-visible spectrophotometer (UV-1800 240 V, Shimadzu corporation, Koyoto, Japan) with 300 to 700 nm wavelength scanning range. The formation of KS@GNPs was confirmed by the Fourier transform infrared spectrophotometer (FTIR) (Perkin-Elmer Model 1600; Norwalk, CT, USA). Here, a pure KBr pellets (2 mg of GNPs were mixed with KBr) was used with the scanning range from 400 to 4000 cm-1. The crystallinity of KS@GNPs was analyzed by X-ray diffractometer (X’pert PRO MPD, PANalytical BV, Netherlands) using an operating voltage 45 kV; a current of 40 mA, Cu radiation (1.54430 Å), and at a scanning rate 0.388/min within the region of 2 thetas between 5 and 90 degrees. The average particle size distribution and the surface charge were determined through a dynamic light scattering (DLS) equipment (Zeta plus 90, Brookhaven Instrument Co., USA). The size, morphology, Energy Dispersive X-ray (EDX) spectrum, and selected electron diffraction (SAED) of KS@GNPs were evaluated by the high-resolution mood using HR-TEM (LEO-912AB OMEGA, LEO, Germany). A thin coat of the sample was prepared for TEM analysis by diffusing a drop of KS@GNPs solution on the cupper grid (operating voltage 200 eV).

**Cytotoxicity and anti-cancer activity of KS@GNPs**

Using the water-soluble tetrazolium (WST) assay kit, the cytotoxicity (NIH3T3 cells) and anti-cancer (LN-229 cells) activity of KS@GNPs were analysed. In short, both NIH3T3 and LN-229 cells were cultured under DMEM medium (incorporated with PS in humidified 5% CO2 incubator at 37 oC for 24 h). After the incubation, NIH3T3 (5×104 cells), and LN-229 (1×105 cells) were separately seeded in the 96 well plates and incubated. Later, based on the cells’ confluence (80-90%), KS@GNPs (0.3125, 0.625, 1.25, 2.5, 5, and 10 µg/mL) was treated to the seeded cells and placed for incubation again. Finally, EZ-CyTox reagent (10 µl) was induced to each well and the absorbance was measured at 450 nm. The cytotoxicity of NIH3T3 and cell viability of LN-229 were determined from the absorbance (OD) using the established formula as described elsewhere [26]. The untreated cells (without KS@GNPs treatment) were regarded as control and denoted as CK.

## Abstract

The combination of green-nanotechnology and biology may contribute to the anticancer therapy. In this regard, using gold nanoparticle (GNPs) as therapeutic molecules can be a promising strategy. Herein, we proposed a novel biocompatible nanogold constructed by simply microwave-heating (MWI) $Au^{3+}$ ions and kenaf seed (KS) extract (as reducing and supporting agent) within a minute. The pathways of gold nanoparticles (KS@GNPs) synthesis were optimized by varying KS concentration ($\lambda_{max}$ 528 nm), gold salt amount ($\lambda_{max}$ 524 nm), and MWI times ($\lambda_{max}$ 522 nm). TEM displayed spherical shape and narrow size distribution (5-19.5 nm) of KS@GNPs whereas DLS recorded Z-average size of 121.7 d.nm with a zeta potential of -33.7 mV. XRD and SAED ring patterns confirmed high crystallinity and crystalline face centered cubic structure of gold. FTIR explored OH functional group involved in $Au^{3+}$ ions reduction followed by GNPs stabilization. KS@GNPs exposure to RAW 264.7 and NIH3T3 cell lines did not induce toxicity while dose-dependent overt cell toxicity and reduced cell viability (26.6%) was observed in LN-229 cells. Furthermore, the IC50 (18.79 µg/mL) treatment to the cancer cell triggered cellular damages, excessive ROS generation, and apoptosis. Overall, this research exploits a sustainable method of KS@GNPs synthesis and their anticancer therapy.

## Results

### Graphical abstract

**Figure 1. Schematic representation of gold nanoparticles synthesis and their biocompatibility and anti-cancer potentials.**

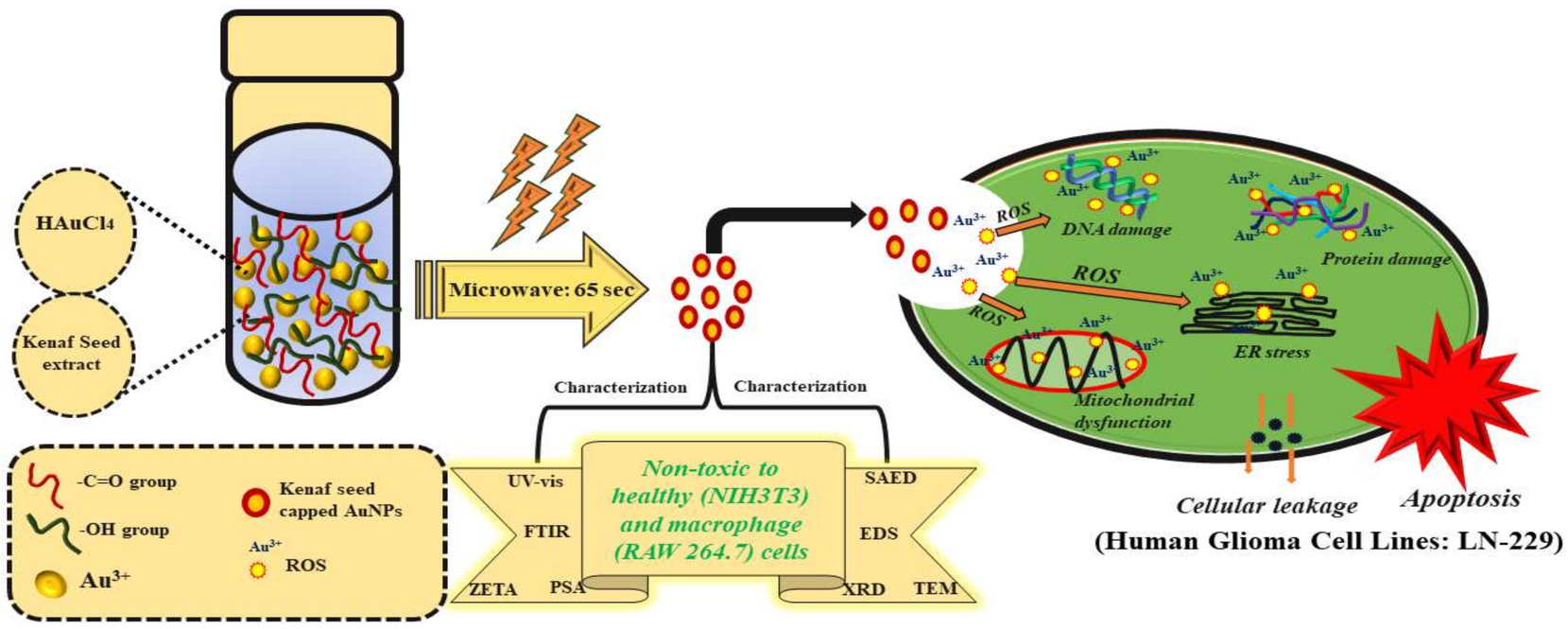



**Figure 2. UV-visible spectra of kenaf seed (KS) stabilized GNPs (KS@GNPs). (A) Formation of KS@GNPs evidenced after color transformation from light white to brick red. Effect of (B) KS concentration (0.1 to 1%), (C) HAuCl4 concentration (0.1 to 1 mM), and (D) microwave irradiation time (0.5 to 1.5 min).**

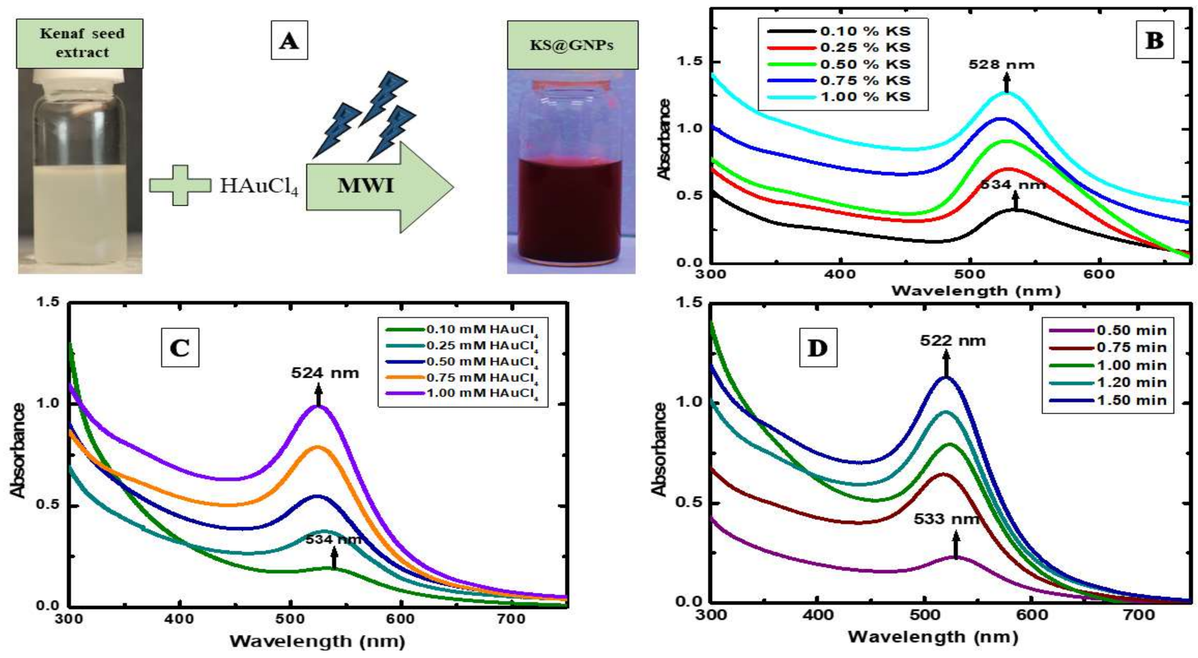
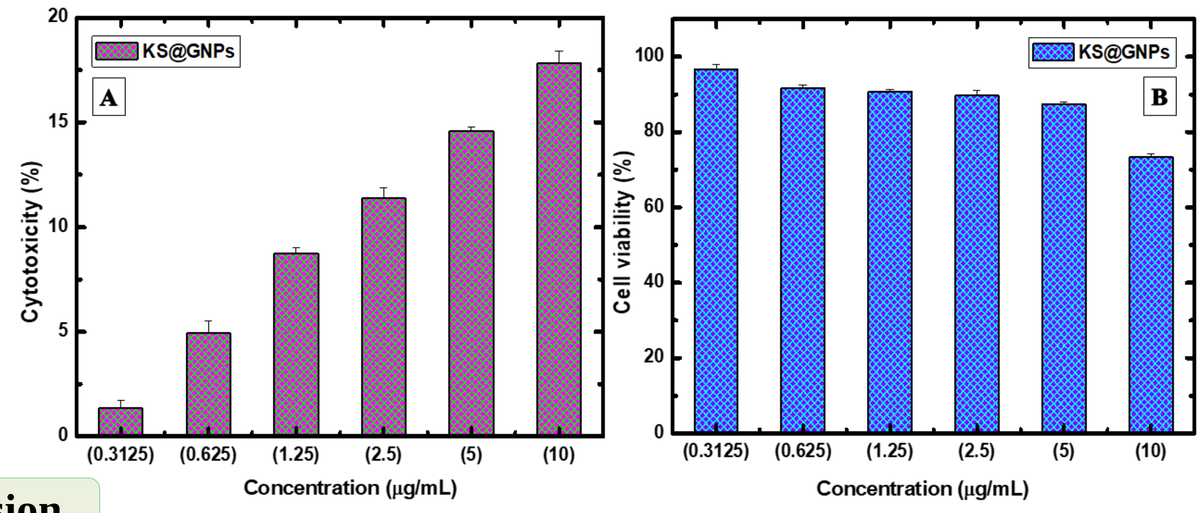



**Figure 3. Cytotoxicity of KS@GNPs in normal mouse fibroblast NIH3T3 cells (A), cell viability of KS@GNPs in human in human glioma (LN-229) cell line (B). Inhibitory concentration (IC50) values of normal (NIH3T3) cells and cancer (LN-229) cells (C).**




## Conclusion

In summary, we have evidenced the effectiveness of in-situ kenaf-seed mediated microwave-assisted uniform gold nanoparticle (GNPs) synthesis. The overall synthesis processes are accurate, inexpensive, and reliable from both technical and recyclable analytical platforms. Here, functional groups of metabolites such as hydroxyl groups from kenaf seed played a pivotal role of both reducing and supporting agent that phenomenon made the kenaf seed as a sustainable material. The obtained KS@GNPs manifested superior characteristics in terms of high-quality crystal, spherical in shape, amorphous, enhanced colloidal stability, and no agglomeration. KS@GNPs exposure to NIH3T3 and (LN-229) cell lines showed utmost relevance and triggered cancer cell death which pinpointed their biocompatibility and anticancer activity.

## Reference

C.D. Adnan, M., Azad, M. O. K., Madhusudhan, A., Saravanakumar, K., Hu, X., Wang, M. H., & Ha, Simple and cleaner system of silver nanoparticle synthesis using kenaf seed and revealing its anticancer and antimicrobial potential, Nanotechnology. 31 (2020) 265101.