

Anticancer Activity of Biosynthesized Silver Nanoparticles using *Murraya koenigii* Leaf Extract against HT-29 Colon Cancer Cell Line

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Abstract

Plants extract from *Murraya koenigii* was used for the synthesis of silver nanoparticles (Ag NPs) using silver nitrate solution. Ag NPs were characterized by UV-vis spectrophotometer, scanning electron microscope (SEM), Energy Dispersive Spectroscopy (EDX) and Fourier Transform Infra-Red Spectroscopy (FT-IR). The formation of stable silver nanoparticles reduced to the colloidal solution are observed by UV-vis spectrophotometer analysis. SEM determination of the brown coloured samples with well dispersed nanoparticles seen after treatment with silver nitrate showed the presence of silver nanoparticle whereas the EDX analysis performed is to confirm the presence of silver molecules in the sample and FTIR measurement carried out identifies the biomolecules present in *M. koenigii* leaf responsible for capping leading to efficient stabilization of the silver nanoparticles. The anticancer potential of the nanoparticles was evaluated using MTT assay on HT-29 colon cancer cell line. Ag NPs showed potent cytotoxic activity against the human colorectal adenocarcinoma (HT-29) cell line at higher concentrations. This study insights the *M. koenigii* synthesized silver NP's could be an effective applicability drug candidate for colon cancer.

Keywords: Plants extract; *Murraya koenigii*; Silver nanoparticles; Colon cancer and HT-29

Introduction

Cancer, a disease of uncontrolled growth of the cell, inhabits all anatomical structures of human beings and changes their phenotypic and physiological condition. In human is due to the accrual of genetic alterations/mutations Bienz and Clevers [1]. Tobacco usage remains as the first and most important identified risk factor for cancer. It is now known that over one-third of cancers are preventable, and one-third potentially curable provided they are diagnosed early in their course [2]. Colorectal Cancer (CRC), one type of cancer that affects the colon of the human. Based on the pathways, including chromosomal and microsatellite instability, CpG island methylator phenotype, genomic mutations, micro RNA and epigenetic changes Kanthan [3] were utilized for the formation and development of CRC Ewing [4] stated that, CRC was due to aggregation of mutations at the genetic level. In India, it was estimated that, 4.4 and 4.1 men per lakh were affected by the colon and rectal cancer, respectively, according to annual incidence rate (AAR). It was also estimated that, prevalence of colon cancer was 3.9 per one lakh women. In the ranking, colon and rectal cancer occupied 8 and 9th position in men. It is the third most common cancer in men (6,63,000 cases, 10.0% of all cancer cases) and the second most common in women (5,71,000 cases, 9.4% of all cancer cases) worldwide. In India, the annual incidence rates (AARs) for colon cancer and rectal cancer in men are 4.4 and 4.1 per 1,00,000, respectively. The AAR for colon cancer in women is 3.9 per 1,00,000. In the 2013 report, the highest AAR in men for CRCs was recorded in Thiruvananthapuram - 4.1 followed by Bangalore - 3.9 and Mumbai - 3.7. The highest AAR in women for CRCs was recorded in Nagaland - 5.2 followed by Aizwal - 4.5 Sirohi [5].

A nanoparticle, the nucleus of nanotechnology, has been vastly being employed for the effective control of cancer cell lines as prominent therapeutic agents. There are number of synthesis procedure has been emerged so far but use of flora-based extracts for nanoparticle synthesis have been more advantageous than microbial process Narayanan and Sakthi [6] and it's an ideal candidate for large scale production [7,8]. Rajendra Prasad, Chang, Guerriero demonstrated the anti-

carcinogenic potential of caffeic acid against different cancer cell lines [9-11]. Devi and Bhimba [12] reported the anticancer properties of *Ulva lactuca* against human cancer cell line such as Hep2, MCF7 and HT-29. Moorthi evaluated bioactivity of silver nanoparticle synthesized from *Sargassum muticum* [13].

P53 gene located in chromosome 17p is found in up to 75% of cases of colorectal cancer. It is a tumor suppressor gene encodes a nuclear protein that functions as a transcription factor. p53 is regarded as one of the most important tumour suppressor genes with a key number of cellular functions such as DNA damage repair, initiation of programmed cell death and cell cycle checkpoint control.

Loss of material from the long arm of chromosome 18 is found in approximately 70% of cases of colorectal cancer and 50% of adenomas. The 18q segment contains three candidate tumour suppressor genes: DCC (deleted in colon cancer gene) and Smad 2 and Smad 4 (initially described as *DPC4*) genes. These proteins are involved in signalling from TGF- β receptors and regulate transcription of key target genes such as c-myc, CBFA1, FLRF and furin McDermott [14].

Materials and Methods

In an Erlenmeyer's flask, 95 mL of 1 mM Silver nitrate (AgNO_3) solution was prepared using chemical synthesis method, and the characterization of silver nanoparticles was performed in sequence using UV-Spectrophotometer, Scanning Electron Microscope (SEM).

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The morphological and chemical nature of the NPs has been identified by using instrumentations such as X-ray diffraction etc. A fraction of aqueous extract of *M. koenigii* was subjected to phytochemical screening as described by Harborne [15] to find presence of Carbohydrate, Coumarins, steroids, Phytosterols, Triterpenoidalsapogenins, Xanthoproteins, reducing sugar, saponins, Tannins, Alkaloids. The Cytotoxic Assay screening was performed using MTT assay as per Mosmann [16] method to test the cytotoxicity of *M. koenigii* synthesised silver nanoparticles against HT-29, the colon cancer cell line.

Synthesis of silver nanoparticles using the curry leaf, *M. koenigii* was carried out in present study. Synthesis of atomic silver was achieved within 15 minutes of incubation at 90°C. The change of the colour of the medium containing aqueous extract of *M. koenigii* and AgNO₃ at the initial as well as in the endpoint was depicted in Figures 1A-1C. The change in the colour from colourless to dark brown was often indicates the excitation of surface plasmon resonance due to reduction of silver nitrate to atomic silver. The light absorption pattern of synthesized nanoparticle was examined using UV-spectrophotometer (JASCO, V630, and Japan) in a range of wavelength from 200 to 800 nm.

The absorption maximum at 428 nm in the UV-spectrophotometer (Figure 2) represents the longitudinal plasmon vibration corresponding to silver nanoparticles. A sharp increase in the intensity of the peak without any shift in the peak position was observed upon varying time. This often evidenced the perfect interaction exists between silver ion

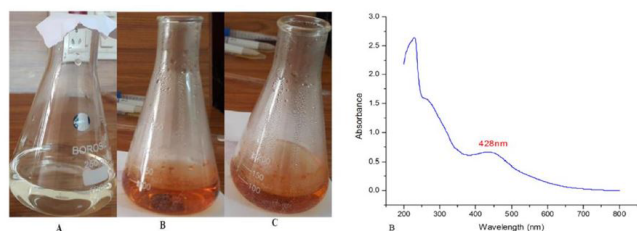


Figure 1: Synthesis of nanoparticles from the leaf extract of *Murraya koenigii*. (A-C) Change of color during reduction of AgNO₃ to Ag. (B) UV-Vis spectra of excitation peak of Ag.

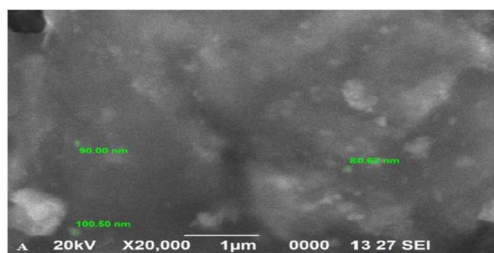


Figure 2: Scanning Electron Microscope (SEM) Image of silver nanoparticles synthesized from *Murraya koenigii* leaf aqueous extract.

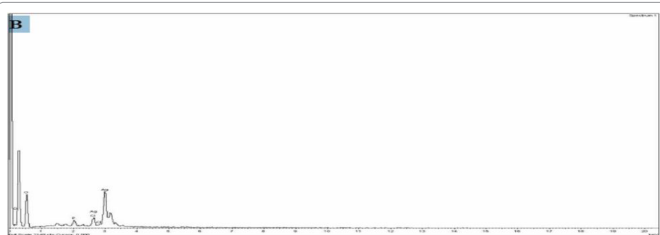


Figure 3: Energy Dispersive X-ray Spectroscopy (EDX) of silver nanoparticles synthesized from *Murraya koenigii* leaf aqueous extract.

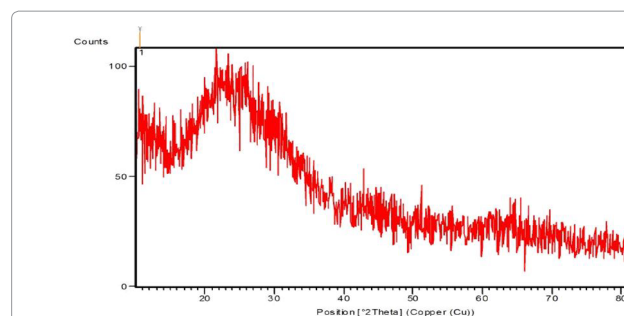


Figure 4: X-Ray diffraction spectra of silver nanoparticles synthesized from leaf extract of *Murraya Koenigii*.

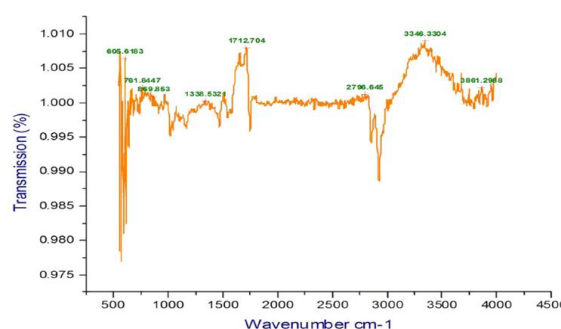


Figure 5: Fourier Transform-Infrared (FT-IR) spectra of silver nanoparticles synthesized from leaf extract of *Murraya Koenigii*.

and phytochemicals of the aqueous extract of the *M. koenigii*. Besides that, their presence has been well defined in SEM image (Figure 3) and was of spherical in shape ranging from 80.62 to 100.50 nm. The presence of silver was confirmed in EDX (Figure 3). In the FT-IR spectrum (Figure 4), intense peaks were observed for silver nanoparticles at 605.6183 cm⁻¹, 761.84 cm⁻¹, 869.85 cm⁻¹, 1338.53 cm⁻¹, 1712.70 cm⁻¹, 2796.64 cm⁻¹, 3346.33 cm⁻¹ and 3861.29 cm⁻¹. The cytotoxic assay of silver nanoparticles against HT-29, the colon cancer cell line revealed excellent anticancer potential of the nanoparticles and the IC₅₀ value was 26.05 μg/mL.

Discussion

The current study revealed that, the synthesis of silver nanoparticle was morphologically visualized in UV-Vis spectrum (Figure 2), with a potent peak observed at 428 nm, which is a characteristic of Ag nanoparticle and is confirmed in SEM analysis (Figure 3). Philip observed excitation at 432, 413 and 411 nm for colloids S1, S2 and S3, respectively. A similar study by Sajeshkumar [17] also reported that similar excitation at 435 nm for *M. koenigii* and also observed spherical shaped nanoparticles in SEM. Logeswari [18] observed maximum peak at 420 nm for silver nanoparticles synthesized by *Ocimumtenuiflorum*, *Solanumtricotatum*, *Syzygiumcumini*, *Centellaasiatica* and *Citrus sinensis*.

FTIR measurements were carried out to identify the possible biomolecules in *M. Koenigii* leaf responsible for capping leading to efficient stabilization of the silver nanoparticles. The IR spectrum (Figures 4 and 5) of silver nanoparticles manifests prominent absorption bands located at 605.6183 cm⁻¹, 761.84 cm⁻¹, 869.85 cm⁻¹, 1338.53 cm⁻¹, 1712.70 cm⁻¹, 2796.64 cm⁻¹, 3346.33 cm⁻¹ and 3861.29 cm⁻¹. Philip [19] observed the IR spectrum of silver nanoparticles at 3412, 2918, 2849, 1636, 1539, 1454, 1375, 1233, 1154 and 1031 cm⁻¹.

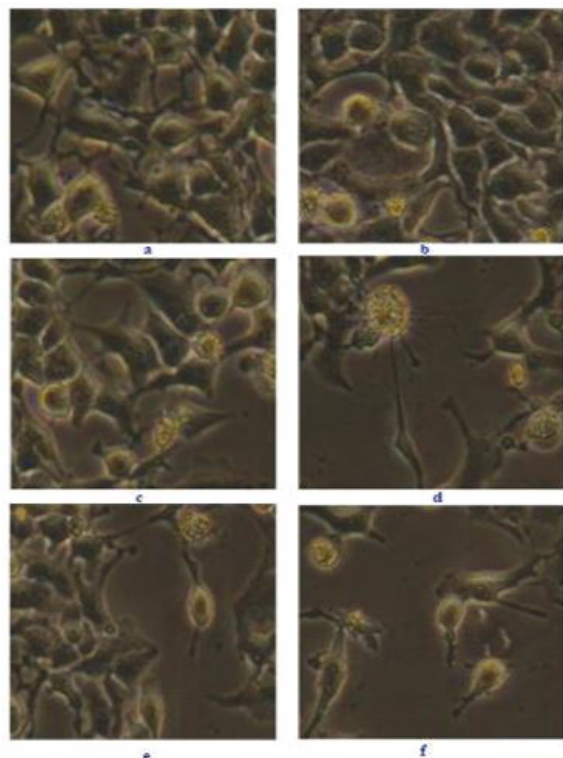


Figure 6: Cytotoxic activity of silver nanoparticles synthesized from leaf extract of *Murraya Koenigii* against HT=29, the colon cancer cell line. a: Control; b-f: HT-29 colon cancer cell line treated with (1.88, 7.5, 15 and 30µg) different concentrations of silver nanoparticles. nthesized from leaf extract of *Murraya Koenigii*.

The nanoparticle synthesized was subjected for its anticancer activity against HT 29 (Colon cancer cell line) and found IC₅₀ value of 26.05 µg/mL and almost 80% reduction in cell survival was noticed during the investigation [20].

The anticancer activity of Ag Nps was highly supported by the works of Prabhu [21]. In the present study, IC₅₀ value obtained was low compared to Nayak [22] who reported IC₅₀ value of 82.39, 83.57 and 78.78 µg/mL by AgNps synthesized from *Cucubita maxima*, *Moringaoleifera* and *Acoruscalamus* respectively. In contrast to Nayak et al. (2015), the present study reported the potent anticancer activity of leaf extract synthesized nanoparticle (Figure 6) [23-26].

The cytotoxicity of silver nanoparticles was due to introduction of reactive oxygen species (ROS) which enunciate the apoptosis pathway and its well established mitochondrial interaction. Besides, emanation of oxidative stress pronounced the genotoxic stress as well as *p53* gene up regulation Setyawati [27] which initiate the apoptosis. It has greatly supported the recommendation of nanomaterials for the anticancer studies. Hence, it was observed from the present investigation that, the cytotoxic activity of silver nanoparticle synthesized from curry leaf samples. It would be further studied for its mode of penetration and suppression of cancer cell line and regulation of genes of guardian of cells.

Conclusion

The rapid biological synthesis of silver nanoparticles using *M. koenigii* leaves extract provides eco-friendly, simple and efficient route for synthesis of benign nanoparticles. The results show that *M. koenigii*

leaves play key roles in the reduction and stabilization of silver to silver nanoparticles. Physical characteristics of silver nanoparticles, such as shape and size, are important for reducing free radicals and cancer cell toxicities. The synthesized particles ranged in size from 80.62 to 100.50 nm and were spherical in shape, as shown by the SEM analysis and the presence of silver was confirmed in EDX. This characterisation is of use for large scale silver nanoparticle production, and could result in economic viability, as well as being eco-friendly for cancer treatment, drug delivery, sensors and commercial appliances and other medical and electronic applications. Silver nanoparticles might be useful for the development of newer and more potent antioxidants and anticancer agents. The biologically synthesized silver nanoparticles could be of immense use in medical field for their efficient anticancer function.

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