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COUROUPITA SP. ENHANCED SILVER NANOPARTICLES AS POTENT ANTICANCER AGENT

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ABSTRACT: Couroupita sp. flower extract enriched silver nanoparticle was synthesized in the present investigation. It was characterized by its excitation at 428nm which was observed in UV-Vis spectrophotometer. Their spherical shaped presence was been clearly noticed in SEM image. In the FT-IR spectrum intense peaks were observed for silver nanoparticles at 975.93, 1637.48 and 3348.25 cm^{-1}. The cytotoxic assay of silver nanoparticles against MCF-7, the breast cancer cell line revealed excellent anticancer potential of the nanoparticle and the IC_{50}value was 164.69µg/mL. Thus, it reveals the anticancer potential of AgNps enriched with flower extract which will be further studied for its cancer therapy.

Keywords: [Couroupita sp. MCF-7, breast cancer cell line, SEM, FT-IR.]

1. INTRODUCTION

Cancer, the disease of multifactorial cause, is characterized by its uncontrolled growth. Invisible and inter organ perching nature made this disease remain one of the dominant among the lethal disease of human beings. 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 (Nair et al., 2005). Globocan (2012) estimates a substantive increase to 19.3 million new cancer cases by 2025. The American Cancer Society estimates that 1, 36,830 people will be diagnosed with colorectal cancer and 50,310 people will die from the disease in 2014 (Alteri et al., 2014). On the Indian scenario, 1.1 million new cancer cases were estimated contributing to 7.8% of global cancer burden (Saranath and Khanna, 2014). Breast cancer is the second leading cause of cancer death among women in the U.S. An estimated 39,620 breast cancer deaths and 2, 32, 340 new cases are expected among women in 2013. Breast cancer is the second leading cause of cancer death among women in the U.S. An estimated 39,620 breast cancer deaths and 2, 32, 340 new cases are expected among women in 2013. So such a deadliest disease treatment with side effect free control is still under search by the researcher all over the world. In support to the essential healthcare field, nanotechnology, now become promising novel solution supporting the healthcare and still bending to help for the welfare of human beings.

Nanoparticles, the nucleus of nanotechnology, have been widely applied 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 Sakthivel, 2010) and it is

promising one for large scale production (Iravani, 2011; Akhtar et al., 2013). Rajendra Prasad et al, (2011); Changet al, (2013); Guerriero et al, (2011) demonstrated the anti carcinogenic potential of caffeic acid against different cancer cell lines. Devi and Bhimba (2012) reported the anticancer properties of Ulva lactuca against human cancer cell line such as Hep2, MCF7 and HT-29. Moorthi et al. (2015) evaluated bioactivity of silver nanoparticle synthesized from Sargassum muticum. Kumara Swamy et al. (2015) and Bupesh et al. (2016) reported the anticancer activity of silver nanoparticles synthesized from Vitex negundo, Lepta deniareticulate and Terminalia chebula against colon cancer cell line respectively. Hence nanoparticles from phytochemical origin could be promising field to manipulate the nanoparticles for the efficient control of cancer cell lines. This boosted the present study to execute the synthesis of silver nanoparticle from Couroupita sp. flower extract and evaluate them for their anticancer activity against breast cancer cell lines.

2. MATERIALS AND METHODS

2.1 Preparation of aqueous seaweed extract

Couroupita sp. was collected from Kalveerampalayam, Coimbatore, Tamil Nadu, India. Its flower was surface sterilized with tap water, shade dried for 15 days and powdered using mixer grinder. Later, aqueous extract was prepared by dissolving 100 mg of powdered Couroupita sp. flower in 100 ml of sterile distilled water. The extract was heated at 80-100 °C for 20 mins, filtered through Whatmann No. 1filter paper and used throughout the study.

Synthesis and characterization of silver nanoparticles (AgNPs)

In an Erlenmeyer's flask, 95ml of 1mM Silver nitrate ($AgNO_3$) solution was prepared using distilled water and 5 ml of aqueous Couroupita sp. extract was added. The reaction mixture was gradually heated to 90 °C for 20 mins and the change in color was envisaged as the evidence of bioreduction. The silver nanoparticle (AgNps) synthesized was characterized for its anticancer application.

2.2. Cytotoxic Assay

The cytotoxic effect of AgNps synthesized using aqueous extract of Couroupita sp. was tested against Human MCF-7, the breast cancer cell lines by using MTT assay followed by Mosmann (1983).

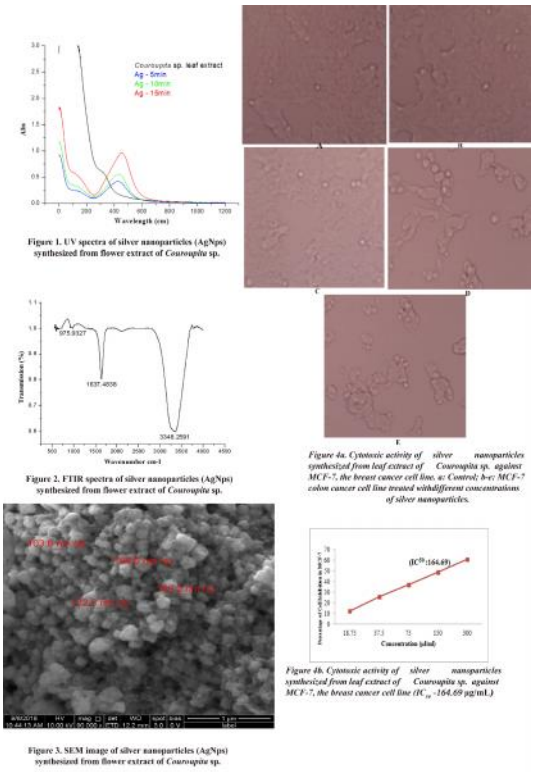

Figure 1. UV spectra of silver nanoparticles (AgNps) synthesized from flower extract of *Couroupita* sp.

Figure 2. FTIR spectra of silver nanoparticles (AgNps) synthesized from flower extract of *Couroupita* sp.

Figure 4a. Cytotoxic activity of silver nanoparticles synthesized from leaf extract of Couroupita sp. against MCF-7, the breast cancer cell line. a: Control; b-e: MCF-7 colon cancer cell line treated withdifferent concentrations of silver nanoparticles.

Figure 3. SEM image of silver nanoparticles (AgNps) synthesized from flower extract of *Couroupita* sp.

Figure 4b. Cytotoxic activity of silver nanoparticles synthesized from leaf extract of Couroupita sp. against MCF-7, the breast cancer cell line (IC_{50} -164.69 µg/mL)

3. RESULTS

Aqueous extract of Couroupita sp. was used for the synthesis of AgNps in present study. Synthesis of atomic silver was achieved within 15 minutes of incubation at 90°C. The change in the color of the medium at the initial as well as in the endpoint signals the AgNps synthesis and was depicted in figure 1. The change in the color from colorless 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 AgNps was examined using UV-spectrophotometer in a range of wavelength from 200 to 800nm. The absorption maximum at 428nm in the UV-spectrophotometer 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. Besides that, their presence has been well defined in SEM image (Figure 3). In the FT-IR spectrum (Figure 5), intense peaks were observed for silver nanoparticles at 975.93, 1637.48 and 3348.25 cm^{-1}. The cytotoxic assay of silver nanoparticles against MCF-7, the breast cancer cell line revealed excellent anticancer potential of the nanoparticle and the IC_{50}value was 164.69µg/mL(Figure 4a & b).

Discussion

The present investigation studied the utilization of flower extract of the Couroupita sp. for the synthesis of silver nanoparticles. This study revealed that, the synthesis of silver nanoparticle was morphologically visualized in UV-Vis spectrum (Fig. 1), with a potent peak observed at 428nm, which is a characteristic of Ag nanoparticle and is confirmed in SEM analysis (Fig 3). Philip et al. (2011) observed excitation at 432, 413 and 411 nm for colloids S_1, S_2 and S_3, respectively. A similar study by Rajeshkumar et al. (2016) also reported that similar excitation at 415-420 nm for C. quianensis which was similar to the results of the present investigation**.** Logeswari et al. (2015) observed maximum peak at 420 nm for silver nanoparticles synthesized by Ocimum tenuiflorum, Solanum tricobatum, Syzygium cumini, Centella asiatica and Citrus sinensis. FTIR measurements were carried out to identify the possible biomolecules in Couroupita sp. flower responsible for capping leading to efficient stabilization of the silver nanoparticles. The IR spectrum (Figure 2) of silver nanoparticles manifests prominent absorption bands indicating the presence of (1637) –C=C- stretch of alkynes. Geetha et al. (2013) and Rajesh Kumar et al. (2016) confirmed the presence of this stretch in her experiment when using Couroupita sp. A close C-N stretching vibrations of aliphatic amines were also at 975 was noticed. An intense peak at 3348 indicates the O-H stretch. It was also a similar peak represented by Geetha et al. (2013) with Couroupita sp. Thus it confirms the presence of phyto-capped AgNps used in the present investigation.

The nanoparticle synthesized was subjected for its anticancer activity against MCF-7 (breast cancer cell line) and found IC_{50} value of 164.69µg/mL (Figure 4a & b) and almost 60% reduction in cell survival was noticed during the investigation. In the present study, IC_{50} value obtained was relatively high compared to Nayak et al (2015) who reported that Cucubita maxima, Moringa oleifera and Acorus calamus energized AgNps exhibited excellent anticancer activity (IC_{50} value of 82.39, 83.57 and 78.78µg/mL respectively). Asharani et al (2009), Sanpui et al. (2011) reported that, 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. It has greatly supported the recommendation of nanomaterials for the anticancer studies. Hence, it was observed from the present investigation that, the nanoparticle synthesized from Couroupita sp exhibited anticancer activity against MCF-7, while its other target cell lines would be studied and will compared for the effective utilization of this AgNps. It would also be further studied for its mode of penetration and suppression of cancer cell line and regulation of genes of guardian of cells.

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