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The impact of anticancer activity upon *Beta vulgaris* extract mediated biosynthesized silver nanoparticles (ag-NPs) against human breast (MCF-7), lung (A549) and pharynx (Hep-2) cancer cell lines

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Highlights

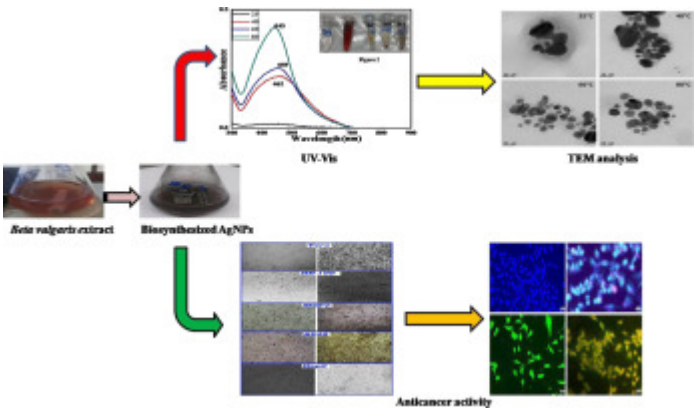
- Phytosynthetic (*Beta vulgaris*) method of producing silver nanoparticles (AgNPs) as an eco-friendly
- The SPR peaks of Ag-NPs for different temperatures shown at ~445 to 465nm
- Ag-NPs HRTEM depicts spherical, circular & triangular shapes with particles size 5–20nm.

- Biosynthesized Ag-NPs showed toxicity towards cancer cell line while no impact on normal cells.

Abstract

The present study tried for a phyto-synthetic method of producing silver nanoparticles (Ag-NPs) with size controlled as and eco-friendly route that can lead to their advanced production with decorative tranquil morphology. By inducing temperature fluctuation of the reaction mixture from 25 to 80°C the plasmon resonance band raised slowly which had an ultimate effect on size and shape of Ag-NPs as shown by UV–visible spectroscopy and TEM results. The biosynthesized nanoparticles showed good cytotoxic impact against MCF-7, A549 and Hep2 cells compared to normal cell lines. Compared to control plates, the percentage of cell growth inhibition was found to be high with as concentrations of Ag-NPs becomes more as determined by MTT assay. The AO/EtBr staining observations demonstrated that the mechanism of cell death induced by Ag-NPs was due to apoptosis in cancer cells. These present results propose that the silver nanoparticles (Ag-NPs) may be utilized as anticancer agents for the treatment of various cancer types. However, there is a need for study of *in vivo* examination of these nanoparticles to find their role and mechanism inside human body. Further, studies we plan to do biomarker fabrication from the green synthesized plant extract nanoparticles like silver, gold and copper nanoparticles with optimized shape and sizes and their enhancement of these noble nanoparticles.

Graphical abstract



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Introduction

The application of nanotechnology in various fields of science has earned a great concern over the past decades due to advancement of nanoparticles, nanotubes and nanowires over the normal materials. The nanoparticles of noble metals with advancement of new materials have been successfully developed for different purposes in engineering and biological sciences. Among the metals used so far, silver nanoparticles (Ag-NPs) have been intensively studied because of their effective use in drug delivery [1], biomedical [2], [3], [4], textile industries [5], water treatment [6], food industries [7], agriculture [8], catalysis and surface-enhanced Raman scattering (SERS). There are different methods popularly used for the synthesis of silver nanoparticles where the chemical reduction of silver salt precursor is reduced to silver nanoparticles. The reducing agents can be simple like, sodium hydroxide [9] or complex such as; citrate [10], polymer substances [11], [12], [13], borohydride, *N*,*N*-dimethyl formamide [14], sodium borohydride [15], trisodium citrate [16], 2-mercaptobenzimidazole [17], sodium dodecyl sulfate [18].

Among the physical methods include photo chemical method, sono chemical deposition, microwave-assisted, electrochemical method, thermal decomposition in organic solvents, and physical vapor/laser deposition methods [19], [20], [21], [22], [23], [24], [25], [26], [27], [28], [29], [30], [31], [32], [33], [34], [35], [36], [37], [38], [39], [40].

Although the chemical and physical methods are proved as efficient tools for synthesizing Ag-NPs, but their commercial for bulk production may pose a great threat the environment due to use of toxic and hazardous reagents and generation of toxic by-products in some instances. Sometimes these products tend to bind to the Ag-NPs surface and may adversely affect their character and performance [41]. In addition, these methods suffer from disadvantages like, low yield, high-energy supplies, and a need for complicated and inefficient purifications [42]. These factors enforce the modern researchers to create some alternative methods for Ag-NPs synthesis, which could combat with these issues to provide evidence as nontoxic and eco-friendly.

Some of the recently developed green methods utilizing biological materials have proven effective ways for synthesis of nanoparticles. The use of plants for the synthesis of Ag-NPs is in focus of intensive research because of their eco-friendly nature. Moreover, the use of plants boasts of several advantages such as elimination of elaborate processes of maintaining cell cultures, easy scale-up for large-scale synthesis and cost-effectiveness. In addition, plant extracts may act both as reducing agents and stabilizing agents in the synthesis of nanoparticles. Typically, a plant-extract-mediated bioreduction for phyto-

synthesis of silver nanoparticles (Ag-NPs) involves mixing the aqueous extract with silver nitrate solution [43], [44], [45], [46], [47].

The present study have been made an attempt towards a facile and simple green method for synthesis of silver nanoparticles (Ag NPs) using silver nitrate $Ag(NO_3)_2$ solution and concentrate of beetroot (*Beta vulgaris*) as the reducing agent. *Beta vulgaris* belongs to family Chenopodiaceae. The claimed therapeutic use of *Beta vulgaris* includes its antitumor, carminative, emmenagogue, and hemostatic and renal protective properties and is a potential herb used in cardiovascular conditions [48]. It possesses antihypertensive, hypoglycemic, antioxidant properties [60]. The anti-inflammatory and hepatoprotective activities of *Beta vulgaris* has been widely studied [50], [51], [52], [53]. In some studies, the *Beta vulgaris* extract has been demonstrated to be an effective multi-organ tumor suppressing agent in laboratory animals [54], [55]. Thus, the study reveals the synthesis of silver nanoparticles utilizing *Beta vulgaris* and cytotoxicity of biosynthesized silver nanoparticles against MCF-7, A549 and Hep-2 cancer cell lines which is the first demonstration [49], [64].

Section snippets

Materials

Silver nitrate salt ($AgNO_3$) and MTT were purchased from Hi Media Laboratories Pvt. Ltd., India. The three different cancer cell lines like, MCF-7, A549 and Hep-2 cell line were collected from King Institute of Preventive Medicine, ICMR, Chennai, India. The plant material (*Beta vulgaris*) were purchased from the local market in Chennai.

Preparation of the Plant Extract

The identified plant material of *Beta vulgaris* was shade dried and powdered finely by utilizing mortar and pestle. 20g of the concentrate powder was broken down in

Silver Nanoparticle (Ag-NP) Formation

Figure1A shows silver nitrate as colorless solution and the concentrate of *Beta vulgaris* is dark red in color (Fig.1B). Fig.1C shows the color of mixture after adding *Beta vulgaris* extract to silver nitrate solution. As such, the solution became grayish red in color which confirms the reduction of silver nitrate into silver nanoparticles. To discover the silver nanoparticles (Ag NPs) in fluid arrangement UV-Vis spectrophotometer was conducted at different temperatures of [1] 25°C, [2] 40°C, [3]

Discussion

The green synthesis of silver nanoparticles has been much considered in recent years because of its simple and eco-accommodating nature. The *Beta vulgaris* concentrate on addition with silver nitrate solution produced the change in reaction mixture from red to light brown. The change in color of the mixture is due to the reduction of silver particles and excitation phenomenon of surface plasmon resonance (SPR) by the silver nanoparticles. This regulation the color change of reaction mixture

Conclusion

The biosynthesis of nanoparticles using plants (phytosynthesis) is an effective method in developing a rapid, clean, nontoxic, and eco-friendly technology. The reaction temperature can greatly influence rate of reaction and particle characteristics. Our single step method of AgNP synthesis could be a suitable way for large scale production of nanoparticles being simple, time consuming and utilizing natural resources. The size of Ag-NPs was confirmed by UV–visible, XRD and HRTEM analysis which

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