Anticancer Potential of Biosynthesized Silver Nanoparticles: A Review

Ketaki G. Walimbe, Pranjali P. Dhawal, and Shruti A. Kakodkar

ABSTRACT

The field of nanotechnology has emerged as a promising course of study branching out into various biomedical fields such as therapeutics, imaging, and diagnostics. Metallic nanoparticles, specifically silver, are an important area of study due to their multifunctionality and diverse morphological characteristics. Amongst the various methods of synthesis of these multifunctional nanoparticles such as physical methods and chemical, green synthesis is the most suitable method due to its eco-friendly nature, cost-effectiveness, and ease of production. This article summarizes the broad spectrum of applications of green silver nanoparticles in the fields of cancer theranostics, imaging and diagnosis, and drug delivery. Bimetallic nanoparticles with silver as one of its major constituents are also explored to get a clear insight into the numerous prospective developments in the respective field.

Keywords: Anticancer, biosynthesized, eco-friendly, silver nanoparticles, therapeutic.

I. INTRODUCTION

Application of nanoparticles in the field of medicine (e.g., antimicrobials (Maria et al., 2015), biosensing (Varghese et al., 2020), imaging (Mukherjee et al., 2014), bioremediation (Salvadori et al., 2022), cosmetics (Gajbhiye and Sakharwade, 2016), among others) has risen exponentially in the last two decades. Nanoparticles are particles whose size ranges from 1-100 nm (Tiquia-Arashiro and Rodrigues, 2016). They have emerged as a very important aspect of modern medicine, as their applications range from imaging to carriers for gene delivery in individual target cells (Annu et al., 2018). Several nanoparticles have multifunctional characteristics such as iron oxide nanoparticles, which are useful in intrinsic imaging as well as in drug delivery (Sharma et al., 2016; Kang et al., 2015).

Theranostics, a novel integration of ‘diagnosis’ and ‘therapeutics’ is emerging as a safe, targeted, and efficient pharmacotherapy that focuses on patient-centred care. The term ‘Theranostics’ was coined by John Funkhouser. It is a new field of medicine that delivers a transition from conventional medicinal practices to the contemporary personalized and precision medicine approach. Theranostics deals with a specially tailored treatment plan based on the uniqueness of each individual and hence, aids in the prescription of the right drug and treatment protocol for patients under investigation yielding a cost-effective and efficient treatment approach (Jeelani et al., 2014).

To incorporate the versatile benefits of nanoparticles in theranostics, ‘theranostic nanoparticles’ were developed. These ‘theranostic nanoparticles’ have a wide range of applications in the diagnostic arena that includes optical imaging, magnetic resonance imaging (MRI), ultrasound, computed tomography, and nuclear imaging (single-photon computed tomography and positron emission tomography) (Zavaleta et al., 2018). Current nanoparticle technologies in the therapy area include drug delivery in cancer patients by liposome-based nanomaterials, neurodegenerative disease therapy, HIV/AIDS therapy, ocular disease therapy, and respiratory disease therapy (Annu et al., 2018). In an ideal scenario, such theranostic nanoparticles should accumulate rapidly and particularly in targeted tissues, efficiently carry out drug delivery without damaging other organs and clear out from the body after a suitable period as nontoxic by-products. This trend of combining therapy and diagnostics is leading to greatly improved personalized disease
management (Abel et al., 2015). The process can be optimized by building more compatible and elaborately designed nanosystems to improve targeting strategies. Theranostics that utilize particles at the nanoscale level have been devised to fabricate a single agent that is a combination of two radioactive drugs; one used for diagnosing the disease and the other used for delivering specialized target therapy to initiate treatment (Zavaleta et al., 2018).

Various types of nanoparticles including gold, platinum, iron, and iron oxides amongst many others have been recognized for their role in different biomedical functions. The rapid and broad range development and the improved practical potential of metallic nanoparticles specifically, has made them an excellent choice in therapeutics, especially in the field of diagnosis, imaging, among others (Azizi et al., 2017). Bio-synthesized silver nanoparticles (b-AgNPs) have been the focal point of several research studies due to their display of versatility and multifunctional biological activities (Mukherjee et al., 2014). Silver nanoparticles exhibit an optical effect that depends on the shape and size of the nanoparticles called localized surface plasmon resonance (LSPR) which enables them to have a range of applications (Abel et al., 2017; Kelly and Johnston, 2014). Silver nanoparticles of small sizes have a very large surface area to volume ratio that allows the attachment and coordination of a large number of ligands, making them a suitable catalyst (Cassano et al., 2018). b-AgNPs exhibit enhanced antibacterial and anti-cancer activities towards cancer cells like human lung cancer cell lines, mouse melanoma cell lines, and human breast cancer cell lines among others. These nanoparticles also exhibit biocompatibility towards multiple human cells making them a suitable drug delivery vehicle. b-AgNPs have been shown to display a unique bright red fluorescence that aids in the process of diagnosis and imaging by detecting the localization of drugs in oncogenic cells and tumor cells (Mukherjee et al., 2014). Along with their biomedical versatility, silver nanoparticles are also more economical than gold nanoparticles, as the cost of production is low and accessibility is more. Biosynthesis of nanoparticles involves using novel methods that are non-hazardous to the environment and further reduces the production costs (Parveen et al., 2016). The current review aims to provide comprehensive detail about green synthesized silver nanoparticles and their potential application as theranostic nanoparticles.

II. GREEN SYNTHESIS OF SILVER NANOPARTICLES AND THEIR THERAPEUTIC APPLICATIONS

Nanoparticles are commonly synthesized using three methods viz. (i) Physical method (ii) Chemical method and (iii) Biological method. Chemical methods of nanoparticle synthesis involve using organic and inorganic reducing agents. Physical methods involve techniques like evaporation-condensation and laser ablation for synthesizing nanoparticles. This method has been proven to be beneficial over chemical methods due to the synthesis of uniformly distributed nanoparticles and comparatively less incidence of contamination and environmental pollution as non-toxic solvents are involved (Iravani et al., 2014). Although physical and chemical methods are easier to perform, these methods pose a threat to the environment due to the extensive use of toxic chemicals as reducing and stabilizing reagents that are non-biodegradable (Vanaja et al., 2014). On the other hand, the biological method or green synthesis of nanoparticles uses biological sources as reducing and capping agents. This method exhibits less toxicity and high efficacy (Nethi et al., 2020). Thus, green synthesis is considered to be an attractive approach due to its ease of production, affordability, and eco-friendly nature (Vanaja et al., 2014). This approach utilizes organisms like bacteria, fungi, and plants as a source of reducing agents for synthesizing different metallic nanoparticles such as gold, silver, cadmium, magnetite, copper, lead, palladium, zinc oxide nanoparticles among others (Iravani et al., 2014; Bilal et al., 2017). Among the different biological methods, plant extracts are considered to be a more viable option due to their ease of maintenance, biosafety, high availability, and cost-effectiveness (Parveen et al., 2016; Kadam et al., 2020).

Plant-mediated green synthesis of nanoparticles is a green chemistry approach that unites the concept of nanotechnology with phytochemistry (Parveen et al., 2016). Additionally, the vast availability of plants facilitates the large-scale production of plant extracts which serve as the reducing agent in nanoparticle synthesis. Plant extracts contain polyphenols and protein constituents which are known to have reducing properties. This ensures the least usage of hazardous chemical reducing agents, thereby reducing the level of toxicity involved in synthesizing such nanoparticles (Mukherjee and Patra, 2017). One of the earliest approaches of plant-based synthesis of silver nanoparticles was with Alfalfa sprouts, owing to their ability to absorb Ag from the agar medium and subsequently transfer it to the shoot region of the plant without any change in the oxidation state. Once reaching the shoot region, these Ag atoms would arrange themselves into nanoparticles by uniting in bundles and forming larger constituents (Gardea-Torresdey et al., 2003; Rauwel et al., 2015). Mukherjee et al. (2014) showed that silver nanoparticles could be synthesized from Olax scandens in a single-step process. These b-AgNPs exhibited not only theranostic ability towards cancer cells but also showed anti-bacterial properties and fluorescence facilitated imaging. Seriphidium quettense along with varying concentrations of AgNO3 was utilized for the green synthesis of silver nanoparticles (Sq-AgNPs) which were then evaluated for their anti-bacterial, anti-cancer, anti-fungal as well as biocompatibility. The Sq-AgNPs exhibited anti-bacterial activity towards a wide range of bacteria including Escherichia coli, Klebsiella pneumonia, and Bacillus subtilis. Sq-AgNPs also proved to be highly efficacious in prohibiting the proliferation of human liver cancer cells. The results of the hemolytic assay revealed that these nanoparticles were highly biocompatible even at high concentrations making them ideal drug delivery agents (Oasim et al., 2019). b-AgNPs synthesized from the leaf extract of Butea monosperma showed anti-proliferative activity towards cancer cells like B16F10, MCF-7 and were found to be biocompatible with the normal cells (Patra et al., 2015). Zizyphus xylopyrus bark extract was used to
synthesize silver nanoparticles that could be used as efficient drug delivery systems due to their biocompatibility with the host cells (Maria et al., 2015). The mycelia extract of fungal precursors such as Ganoderma neo-japonicum was also employed for the synthesis of silver nanoparticles to target the cell viability and membrane leakage system of human breast cancer cells. The consecutive treatment of MDA-MB-231 breast cancer cells with varying concentrations of b-AgNPs revealed that the cell viability of the cancer cells was prohibited and cell damage was observed due to membrane leakage induced by these AgNPs (Gurunathan et al., 2013). M. J. Ahmed et al. (2019), tested the therapeutic potential of the plant Jurinea dolomiae against cervical cancer cell lines (HeLa) and breast cancer cell lines (MCF-7). This in vitro study of cytotoxic properties of silver nanoparticles synthesized from the leaf extract of J. dolomiae not only proved its anti-cancer potential but also the antioxidant properties possessed by the plant. The apoptosis study also proved the high biocompatibility levels of these b-AgNPs as compared to Cisplatin, a common chemotherapeutic drug (Ahmed et al., 2019). The anti-cancer potential and apoptotic activity of silver theranostic nanoparticles synthesized using Fagonia indica against human breast cancer cells were studied by Ullah et al. (2020). Different mechanistic assays like Acridine orange/Ethidium Bromide (AO/EB) assay, DAPI assay, and Annexin V/PI assay were used to test and unveil the mechanism of action of these b-AgNPs. It was found that the b-AgNPs triggered the activation of caspases 3 and 9, which caused morphological changes in the plasma membrane of the cancer cells, leading to nuclear condensation followed by apoptosis (Ullah et al., 2020). The wide variety of plants used for the green synthesis of silver nanoparticles exhibits the vast and unexplored potential of these silver nanoparticles in the area of theranostics.

The anti-diabetic and cardioprotective potential of silver nanoparticles synthesized from the plant Syzygium cumini were tested by Atale et al. (2017). The results of this study concluded that S. cumini derived silver nanoparticles were efficacious in suppressing glucose-induced cardiac stress. The mechanism of action was found to be the maintenance of the cellular integrity and reduction in the oxidative stress (Atale et al., 2017).

Zeedan et al. (2020), conducted an in-vivo and in-vitro experiment to test the anti-viral effects of silver nanoparticles synthesized from olive leaves and natural honey. The antiviral efficacy was tested using MDBK cell culture and experimental animals. These green AgNPs were found to protect the MDBK cell culture and the experimental animals from the BoHV-1 (bovine alpha herpesvirus 1) proving their antiviral potential. b-AgNPs synthesized from Oscillatoria sp. and Spirulina platensis were checked for their antiviral property and were found to be highly effective against the Herpes Simplex virus (El-Sheekh et al., 2020).

III. APPLICATIONS OF GREEN SYNTHESIZED SILVER NANOPARTICLES IN CANCER THERAPY

According to a WHO report in 2018, cancer is one of the leading causes of death worldwide (Aziz et al., 2019). Cancer therapy mostly includes surgery, chemotherapy, and radiation therapy and is widely known to have harsh side effects such as increased toxicity, damage of healthy cells, and tumor recurrence (Cherukula et al., 2016). Three of the most common cancers include lung cancer, breast cancer, and colon cancer, of which lung cancer has the highest fatality rate. The primary cause of cancer is uncontrolled cell growth and proliferation induced due to variations in gene expression and subsequent metastasis of the tumor cells to distant tissues and organs (Aziz et al., 2019; Balakumaran et al., 2015). The urgent need for specialized, targeted therapy arises as a result of long-term adverse effects induced by anti-cancer drugs in the systemic circulation and poor prognosis of such treatment procedures (Yhee et al., 2014).

The use of biosynthesized silver theranostic nanoparticles for cancer therapy can prove to be an effective, alternative approach to the conventional methods used for cancer treatment (Cherukula et al., 2016). Nanoparticles possess immense potential in specialized cancer therapy due to their unique physical, chemical, and biomedical properties which, as mentioned earlier, are attributed to their small size, large surface area, and diverse pharmacological properties, for instance, biocompatibility, cancer-killing ability, as imaging facilitators among others (Yhee et al., 2014). Biosynthesized silver theranostic nanoparticles have gained attention due to their role in cancer diagnosis and alternative targeted drug delivery systems (Karmous et al., 2020; Madamsetty et al., 2019). These nanoparticles either damage tumor cells and oncogenes or protect healthy cells from cancerous cells using mechanisms that employ the antioxidative and antitumor properties found in plants (Karmous et al., 2020). The mechanism of action differs in different b-AgNPs based on their plant precursors. This mechanism ranges from cell cycle arrest, inhibition of angiogenesis, membrane damage, and leakage, DNA damage, mitochondrial destruction, misfolding of proteins, apoptotic cell death, among others (Aziz et al., 2019; Gurunathan et al., 2013; Mukherjee et al., 2014; Patra et al., 2015; Ullah et al., 2020; Liang et al., 2015; Kuppusamy et al., 2016). The choice of plant species, type of extract, and concentration of phytochemicals are of prime importance during the synthesis of nanoparticles. A rise in metal concentration or leaching of trace elements from biosynthesized metal nanoparticles can cause an increase in oxidative stress which can, in turn, increase the risk of cancer (Karmous et al., 2020).

A. Role of b-AgNPs as theranostics in Breast Cancer Therapy

Breast cancer, a type of adenocarcinoma, is the second most commonly occurring cancer in the world and also a major cause of cancer deaths among women (Gurunathan et al., 2013; Jamal et al., 2002). MDA-MB-231 and MCF-7 are two of the most commonly studied human breast cancer cell lines (Gurunathan et al., 2013).

Silver nanoparticles have been shown to display promising results in inhibiting cell growth of breast cancer cells in-vitro. S. Dinparvar et al. (2020), b-AgNPs from the seed extract of the plant Cuminum cyminum and checked its efficacy against human breast cancer cell line MCF-7 and human breast metastatic cancer cell line AU565. The action of chemically synthesized AgNPs and b-AgNPs was...
compared by a cytotoxic analysis and an anticancer study. b-AgNPs were not only non-toxic but also exhibited strong inhibitory effects against human breast cancer cell lines at 0.25 and 0.5 μg/ml. Both b-AgNPs and chemically synthesized AgNPs showed strong inhibition against cancer cells, but b-AgNPs proved to be far less cytotoxic than the latter (Dinparvar et al., 2020). Jeyaraj et al. (2013) used Sesbania grandiflora leaf extract as a substitute for a reducing agent while biosynthesizing AgNPs to check their inhibitory action against human breast cancer cell line MCF-7. MTT, AOEB, Hochest, and COMET assays proved the effective anti-proliferative effect of these b-AgNPs against MCF-7. Cell damage was induced by multiple pathways including membrane damage, increase in oxidative stress, and apoptosis (Jeyaraj et al., 2013). In a similar study conducted by Ramar et al. (2015), Solanum trilobatum was used to synthesize silver nanoparticles and tested for in-vitro anticancer effect on MCF-7 cell line and was proven to be extremely effective (Ramar et al., 2015). Cytotoxicity studies conducted on b-AgNPs synthesized from the root extract of Rheum emodi also showed their anti-cancer activity against MCF-7 in a dose-dependent manner (Sharma et al., 2015). Heydari and Rashidipour (2015), used the extract of Oak fruit hull for the preparation of silver nanoparticles and tested their cytotoxicity against MCF-7 cell line. Silver nanoparticles synthesized from Melia dubia leaf extract by Kathiravan et al. (2014), showed phenomenal anticancer activity against human breast cancer cell line KB. Piper longum fruit used by Reddy et al. (2014), to prepare b-AgNPs showed strong anti-cancer activity to the MCF-7 cell line. b-AgNPs synthesized from the aqueous extract of Citrullus colocynthis showed active anti-cancer activity against multiple cancer cell lines including MCF-7 (Shawkey et al., 2013).

Analysis of cytotoxicity of b-AgNPs synthesized from Fagonia indica was conducted using the MTT cell viability assay (3-(4,5-Dimethylthiazol-2-Yl)-2,5-diphenyltetrazolium bromide). The results displayed that the inhibition in the growth of in-vitro cultured breast cancer cells was influenced by the concentration of b-AgNPs. Alteration in nuclear chromatin morphology and apoptosis were studied using the DAPI assay and Annexin V/PI flow cytometric assay. The results showed that the nanoparticle-treated cells had a distinct bright colour, abnormal nuclei, and condensed chromatin with irregular cell structure (Ullah et al., 2020). These results were in accordance with previous research work on the influence of green synthesized theranostic nanoparticles on MCF-7 cell lines (Venugopal and Rather et al., 2017; Bhattacharyya et al., 2008; Kathivaran et al., 2014). The AO/EB assay exhibited the mechanism of action of membrane damage of the MCF-7 cells (Ullah et al., 2020; Venugopal et al., 2017). The b-AgNPs treated cells showed shrinkage, membrane blebbing, and nuclear fragmentation leading to cell damage and subsequent apoptosis (Ullah et al., 2020; Patra et al., 2015). Annexin V/Propidium Iodide apoptosis detection assay conducted by several researchers indicated the possibility of inducible apoptosis via autophagy, mitochondrial dysfunction, lipid peroxidation and arresting of the cell cycle (Ullah et al., 2020; Liang et al., 2015; Venugopal et al., 2017; Kikuchi et al., 2012). Previous studies have shown that b-AgNPs inactivate caspases which are known to be involved in apoptosis and sometimes cause inflammation (Mukherjee et al., 2014). The formation of reactive oxygen species (ROS) induces oxidative stress on the cell which causes malfunction of various cell components, cell cycle as well as DNA damage, endoplasmic reticulum stress conditions, and misfolding of proteins (Mukherjee et al., 2014; Ullah et al., 2020; Kikuchi et al., 2012; Azizi et al., 2017). All the results indicate a positive response towards green synthesized silver nanoparticle therapy against breast cancer cells and a definitive need for further research and development in this field.

B. Role of b-AgNPs as theranostics in Lung Cancer Therapy

Lung cancer, another type of adenocarcinoma, prevails to be a major public health hazard and the most fatal cancer-causing maximum deaths worldwide (Barabadi et al., 2020). Lung cancer screening and diagnosis are usually done with a computerized tomography (CT) scan. Venkatesan et al. (2014) biosynthesized silver nanoparticles from Rosa damascena and checked their efficacy against human lung cancer cell line A549. These nanoparticles were not only hemocompatible but also exhibited anti-cancer activity against human lung cancer, although the mechanism is unclear (Venkatesan et al., 2014). Another study conducted by Khanra et al. (2015), involved the biosynthesis of silver nanoparticles using the leaf extract of Scoparia dulcis and checking its anti-proliferative efficacy against the A549 cell line. Along with having high antimicrobial activity S. dulcis also showed anti-cancer activity against A549 and PA-1 (human ovary cell line) making it a promising therapeutic agent in the future (Khanra et al., 2015). b-AgNPs synthesized from a seagrass Cymodocea serrulata also showed high cytotoxic potential and anticancer activity against A549, making it a candidate as a non-toxic cancer therapy agent (Palanippan et al., 2015). Khanra et al. (2016), used Croton bonplandianum Baill. leaves to prepare AgNPs and evaluate their anti-cancer activity against A549. The MTT assay revealed the strong anti-cancer potential of b-AgNPs and, it was found that the nanoparticles acted by reducing ATP content due to mitochondrial damage. This further increased the production of ROS species leading to a rise in oxidative stress (Khanra et al., 2016). b-AgNPs prepared from Olax scandens also exhibited enhanced anti-cancer activity against the A549 cell line (Mukherjee et al., 2014). One of the notable studies on in-vitro and in-vivo applications of b-AgNPs against lung cancer showed that AgNPs significantly suppressed proliferation of H1299 lung cancer cell lines and tumor growth in xenograft severe combined immunodeficient mouse model. Further, the AgNPs also induced apoptosis in a dose-dependent manner by increasing caspase 3 and surviving levels (He et al., 2016). Silver nanoparticles extracted from Punica granatum exhibited strong anti-cancer activity by cell growth inhibition of the A549 cells (Annu et al., 2018). MTT cell viability assay conducted by various researchers in diverse projects confirmed no cytotoxic effects on normal cell growth and non-proliferative nature for cancerous cells (Annu et al., 2018; Mukherjee et al., 2014; Venugopal et al., 2017). b-AgNPs have different mechanisms of action that enable them to curb the growth of cancerous cells, one of
them being creating oxidative stress and activation of apoptosis pathway by upregulation of the p53 protein (Mukherjee et al., 2014; Mei et al., 2012; Gurunathan et al., 2013; Fridman and Lowe, 2003). DNA damage in A549 cells was also a frequently observed side effect caused due to interruption of the mitochondrial respiratory chain as well as arresting the cell cycle at the G2/M phase (Mukherjee et al., 2014; Annu et al., 2018). Oxidative stress was generated by using H2O2 which helped in measuring the intracellular ROS scavenging that led to the malfunctioning of cellular organelles (Annu et al., 2018). It can be easily concluded that b-AgNPs have huge unexplored potential in the field of lung cancer theranostics.

C. Role of b-AgNPs as theranostics in Colon Cancer Therapy

Colon cancer is the third most common cancer worldwide. Almost 10% of cancer deaths are caused by colon cancer and the diagnosis is confirmed by colonoscopy, stool DNA test, Sigmoidoscopy, or Guaiac based fecal occult blood test. P. Kuppusamy et al. (2016), made use of an aqueous extract of Commelina nudiflora to synthesize AgNPs and study their anti-cancer properties. The b-AgNPs exhibited reduced cell viability coupled with increased cell cytotoxicity in HCT-116 colon cancer cells. In the same study, the mRNA gene expression in the HCT-116 cells was analyzed. Interestingly, it was observed that the expression of pro-apoptotic genes coding for Caspase 3, Caspase 8, and Caspase 9 was more upregulated in HCT-116 cell lines that were treated with the b-AgNPs as compared to those treated with a normal chemotherapeutic drug, Cisplatin (Kuppusamy et al., 2016).

b-AgNPs synthesized from Pimpinella anisum seeds have also showed high cytotoxicity and reduced cell viability in HT115 colon cancers (ALSalhi et al., 2016). Anti-cancer activity of Rosa indica was analyzed against HCT15 colon cancer cells, and was found to be a good anti-cancer agent as it reduced cancer cell viability, Bcl-2 expression, increased Bax levels, and caspase 3 and caspase 9 activities that induce apoptosis (Manikandan et al., 2015). Another study utilized leaf extract of Vitex negundo for synthesizing AgNPs and studied their efficacy against HCT15. The results of the MTT cell viability showed strong inhibition of cancer cell proliferation. The nuclear morphology study using propidium iodide staining showed an increase in apoptosis and cell cycle arrest at G0/G1 and G2/M phases (Prabhu et al., 2013). Gymnema sylvestre was used by Arunachalam et al. (2015), to prepare b-AgNPs to study and analyze their potential anticancer activity against HT29 human colon cancer cell line. They found that once the nanoparticles penetrated the cell by various mechanisms like phagocytosis, pinocytosis, and endocytosis, DNA damage and apoptosis (Arunachalam et al., 2015). All these findings point towards the promising applications of green synthesized silver theranostic nanoparticles in combating cancer.

D. Role of b-AgNPs as theranostics in other forms of cancer

Biosynthesized theranostic AgNPs prepared from a wide variety of plants are also being studied for their role in cancer therapies for many different types of less common cancers. Jeyaraj et al. (2013), used b-AgNPs synthesized from Podophyllum hexandrum to check its cytotoxic efficacy and to study the role of caspases in apoptosis on the human cervical cancer cell line, HeLa. It was found that these nanoparticles could selectively target and inhibit the cell cycle and mechanism of HeLa cells by inducing DNA damage and caspase-induced cell death (Jeyaraj et al. 2013). Heliotropium indicum was used to synthesize silver nanoparticles whose anti-cancer efficacy was checked against HeLa cancerous cells. Results of the MTT assay revealed that these b-AgNPs successfully inhibited the growth of HeLa cells in a dose and time-dependent manner. Their anti-cancer activity was attributed to their amphiphilic and membrane penetrative nature as well as their ability to induce a state of hypoxia in the cells (Vijjistella, 2014). Sukirtha et al. (2012), used Melia azedarach to synthesize b-AgNPs for an in-vitro study of their effect on HeLa cancer cells. The anti-cancer activity of the nanoparticles was found to be in a dose-dependent manner with a lethal dose value of 300μg/ml against the HeLa cell line (Sukirtha et al., 2012). Mishra et al. (2012), studied the anti-cancer effect of Azadirachta indica b-AgNPs on the SiHa cervical cancer cell line. They reported the vast anti-cancer potential of A. indica along with it being a very cost-effective anti-cancer therapeutic agent as well.

Detailed study of laryngeal cancer therapy was also done by researchers. Devi and Bhimba (2012), used the seaweed Ulva lactuca to biosynthesize silver nanoparticles and test their anticancer efficacy against Hep-2 laryngeal cancer cell line. The potential anti-proliferative effect of b-AgNPs against Hep-2 cells was confirmed and was found to be nontoxic to the normal cells (Ahmad et al., 2010). The biomedical potential of the plant Citrullus colocynthis was tested against Hep-2 cell line by Satyavani et al. (2011). These b-AgNPs inhibited the growth of the cancerous cells in a dosage-dependent manner by DNA damage followed by apoptosis (Satyavani et al., 2011). Another very important study involved the use of Suaeda monoica leaf extract to prepare b-AgNPs and testing their anti-cancer potential against Hep-2 cancer cells. A partial reduction in the viability of the cancer cells was induced at a lethal dose value of 500nM. This anti-cancer potential was attributed to the intracellular physicochemical interaction of the b-AgNPs with the proteins and phosphate groups as well as the nitrogen bases in the DNA (Satyavani et al., 2012).

Andean mora leaf was used to biosynthesize silver nanoparticles and its anti-proliferative effect on Hep-G2 human liver cancer cells was checked. This study paved the way for the effective use of b-AgNPs in not only anti-cancer therapy but also drug delivery (Kumar et al., 2016). Studies conducted on the anti-cancer activity of Allium sativum b-AgNPs against Hep-G2 cancer cells found potential cell inhibitory effects against the cancer cells making it a valuable anti-cancer drug in the future (Pandian et al., 2015). Silver nanoparticles biosynthesized using the root extract of Citrullus colocynthis by Shawkey et al. (2013), were used to reveal its anti-cancer activity against multiple cancer types including breast cancer, colon cancer, liver cancer and intestine cancer. All these results prove the extremely vast potential of b-AgNPs in the arena of cancer.
theranostics due to their non-toxic, cost-effective and easily penetrative nature.

IV. APPLICATION OF B-AGNPS IN IMAGING AND DIAGNOSIS

The versatile nature of green theranostic nanoparticles allows them to be used for a wide range of biomedical applications like diagnostic screening, fluorescence imaging, drug delivery, etc. The multifunctional properties possessed by biologically synthesized nanoparticles assist in providing multiple imaging platforms for easy and rapid disease verification and exact localization of tumor cells and damaged tissues. The individual properties of these multifunctional nanoparticles are harbouring by altering the surface properties to match the required application (Ahmed et al., 2012).

Plant extracted organic dye molecules are now being studied and applied for thermal imaging due to their low tissue absorbance and enhanced photothermal effects (Cherukula et al., 2016). The various phytochemicals present in plants and their penetrative nature are now being exploited for their fluorescence abilities. The fluorescent molecules present inside the plant cell complex with b-AgNPs, penetrate the cells when incubated with such b-AgNPs. These b-AgNPs with fluorescent properties can facilitate early diagnostic screening of serious diseases such as cancer. This principle was studied by performing fluorescence imaging of A549 human lung cancer and B16 mouse melanoma cell lines after treating them with b-AgNPS treated with the extract of Olax scandens. The cell lines treated with b-AgNPs displayed red fluorescence which was also the characteristic fluorescence displayed by the phytochemicals present in the Olax extract. Surprisingly, chemically synthesized AgNPs did not display any peculiar red fluorescence (Mukherjee et al., 2014).

He et al. (2013), successfully synthesized b-AgNPs from the extract of Chrysanthemum morifolium and explored the biomedical application of these nanoparticles in clinical ultrasound gel for diagnosis. Diagnosis of a specific ailment using nanoparticles in molecular imaging involves analyzing the correlation of the signal generated with the phenotype. The size of the diseased area, along with the cancer stage and biochemical nature can be analyzed using the location and intensity of nanoparticle signals generated at the target site. An ideal nanoparticle system should possess a central imaging core surrounded by small molecule therapeutic agents. The presence of a cloak of protective polymer can protect these nanoparticle systems from immune cells and, the system, in turn, uses ligands such as IgG for active targeting. Most clinically viable silver nanoparticles are used in magnetic resonance screening while the other types are being modified in terms of their toxicity and biodistribution profiles to enhance their biocompatibility (Jokerst and Gambhir, 2011).

Theranostic nanoparticles and their applications in biomedical fields and cancer theranostics have been ongoing for the past several years, but many challenges remain. One of the major challenges is the formulation of nanoparticles with multi functionalities making the nano-system extremely complicated. To ensure successful application of theranostics nanomedicine, appropriate imaging choice must be made, and considerable in-vivo assessment is highly required (Madamsetty et al., 2019). Although limited research has been performed in the application of green synthesized silver theranostic nanoparticles in imaging and diagnosis, extensive clinical research is still required to unearth its vast undiscovered potential and scope in the future.

V. APPLICATIONS OF GREEN SYNTHESIZED SILVER NANOPARTICLES IN DRUG DELIVERY

Nanoparticle-based drug delivery systems and vehicles (such as nano metal particles, polymers, and biological materials) have been extensively studied due to their diagnostic and theranostic properties (Mukherjee et al., 2014; Ahmad et al., 2010; Jahangirian et al., 2017). Currently, cancer is the most common and widely studied and evaluated disease for nano vehicle-based targeted delivery, followed by neurodegenerative diseases, certain infections, and autoimmune diseases (Yetisgin et al., 2020). The demand for such an efficacious system has increased due to the reduced circulation time and limited target efficiency of the currently available treatments (Xie et al., 2017). An ideal, efficient targeted delivery system includes the vehicle loaded with the drug or delivery agent being retained in the circulatory system of the organism’s body for a desirable amount of time, protecting themselves from the immune system of the body, and lastly releasing the drug at the target site (Yetisgin et al., 2020). Nano-vehicles protect the externally injected chemotherapeutic agents, genes, and imaging agents against the harsh environment in systemic circulation ensuring targeted delivery (Xie et al., 2017). However, it should be noted that such systems need to be experimentally analyzed first for their safety before using them in an in-vivo model, specifically animal models (Mukherjee et al., 2014). Due to their small size and penetrative nature, nanoparticles can easily enter the human body, much faster than larger-sized commercial drugs. Furthermore, the use of biosynthesized nanoparticles for drug delivery poses a lesser risk as compared to the adverse effects caused by toxic pharmaceutics, as no harmful reducing or capping agents are used (Lam et al., 2017). The nanoparticle-based drug delivery systems can be used for the delivery of nontoxic prodrugs to tumor cells and damaged tissues. Cellular stimulus at the target site or damaged tissue converts this prodrug to a toxic form which causes subsequent and selective damage of tumor cells and damaged tissue (Sahoo et al., 2016).

Mukherjee et al. (2014), tested the in-vivo biocompatibility of b-AgNPs based nanocarriers towards different types of normal cells including human umbilical vein endothelial cells (HUVEC), rat cardio myoblast cell line (H9C2), and Chinese hamster cell line (CHO), and used the MTT assay to track their metabolic activities. It was found that b-AgNPs were nontoxic to the cells even at a higher concentration as opposed to chemically synthesized AgNPs, thereby indicating their suitability for in-vivo applications as drug delivery vehicles or carriers.

In another study, polyethylene glycol-silver (PEGylated) decorated graphene nanocomposites (NGO-AgNPs-PEG) synthesized from Azadirachta indica extract were subjected

DOI: http://dx.doi.org/10.24018/ejbio.2022.3.2.338
to a novel syn-graphenization method and studied for their capability as a nano-vehicle for drug delivery against cancer cells. A comparative study assessing the effect of cancer drug, free doxorubicin (DOX) and DOX loaded NGO-AgNPs-PEG on HeLa cancer cells and HaCaT cancer cells were conducted. Based on the drug loading and unloading efficiency study and response to pH stimulus it was seen that the DOX released from the NGO-AgNP-PEGs was 218% more efficient than the free DOX. This proves that these silver nanoparticles could also act as subsidiary carriers or as a supportive driving force for the delivery of the drugs to the selective cells or tissues. The anti-proliferative study on the HaCaT cells showed that the DOX-loaded b-AgNPs were less harmful to normal cells as compared to free DOX. This study elaborates the promising prospects of NGO-AgNP-PEGs in targeted and controlled drug delivery therapies (Palai et al., 2019).

Nano-based targeted delivery systems in infectious disease therapy are mostly used for antimicrobial drugs (Sahoo et al., 2016). Metallic nanoparticles, specifically silver nanoparticles have been used for the in-vitro evaluation of Candida albicans and Escherichia coli with the conjugated drugs being Fluconazole (FLC) and Ampicillin respectively (Yetisgin et al., 2020; Longhi et al., 2015; Brown et al., 2012). Longhi et al. (2015), studied the antifungal effect of silver nanoparticles biosynthesized from Fusarium oxysporum individually and then paired with the antifungal drug FLC. The effect of the b-AgNPs alone was almost negligible but in combination with FLC, the growth of C. albicans was reduced by 16 to 64 times. A dose-dependent decrease was observed in the cell viability of the biofilm proving that b-AgNPs could be used as an effective control agent against microbial diseases causing infection (Longhi et al., 2015).

Optimization of the drug encapsulation process and high duplicability at considerably lower cost are important factors that impact the amalgamation of theranostic nanomedicine in the clinical field (Madamsetty et al., 2019). Green synthesized nanomaterials are also being studied in the field of gel dosimetry for radiotherapy, but there is still a broad field of study yet to be unraveled and explored (Vedelago et al., 2018). In recent years, much encouraging evidence has sprouted that ensures a positive outlook for bio-inspired and green synthesized theranostic nanoparticles and their clinical applications in not only cancer treatment, but a wide range of biomedical applications (Madamsetty et al., 2019).

VI. BIMETALLIC NANOPIERCLES AND THEIR APPLICATIONS

Bimetallic nanoparticles have been gaining recognition and scientific attention for their multifunctionality and superior properties over monometallic nanoparticles. The knowledge and information available about biomedical applications of bimetallic nanoparticles, specifically green synthesized bimetallic nanoparticles, is scarce because of limited research and experimentation being conducted in this respective arena. Despite their ease of production, cost-effectiveness and eco-friendly nature, the mechanisms of synthesis of these nanoparticles have been poorly explored (Duran and Seabra, 2018).

The introduction of a second metal for the formation of a heterogeneous bimetallic structure instantly enhances the attractiveness and the therapeutic potential of the resultant structure as useful properties of both metals have a better effect when used in a combination in comparison to when used singly. The addition of a new metal heavily influences the structural and intrinsic properties of the nanoparticles by the enhancement of the magnetic, optical as well as plasmonic properties, while also maintaining stability (Srinoi et al., 2018). Silver (Ag) and gold (Au) nanoparticles have been thoroughly explored by researchers for their wide range of biomedical and theranostic applications including anti-cancer, anti-microbial, anti-fungal, and antioxidant activities. A combination of the two to form bimetallic nanoparticles has been known to have better biocompatibility and unique properties as compared to that arising from both noble metals individually (Gupta et al., 2020). In the case of Ag-Au nanoparticles, the surface plasmon resonance levels can be altered according to the required structural characteristics just by controlling the Ag: Au ratio in the displacement reaction taking place during the synthesis of this bimetallic structure. This flexibility allows the bimetallic structure to have variable shapes and high optical activity (Srinoi et al., 2018). This section will explore green synthesized bimetallic nanoparticles containing silver and their theranostic applications.

Sivamaruthi et al. (2019) studied the biosynthesis of silver-palladium (Ag-Pd) nanoparticles from the fruit extract of Terminalia chebula. The anti-cancer analysis showed that the nanoparticles acted by inducing the production of ROS which in turn leads to mitochondrial damage and subsequent apoptosis. The antimicrobial study was conducted by checking its effectiveness against the methicillin-resistant bacteria Staphylococcus aureus and Pseudomonas aeruginosa and was found to be efficacious against both species. The cytotoxic studies also showed that these bimetallic nanoparticles were harmless towards the normal body cells and had no undesirable hemolytic effect as well (Sivamaruthi et al., 2019).

Jiang et al. (2019) developed a novel method of synthesis of Ag-Au bimetallic nanoparticles using Escherichia coli and studied their various biomedical applications. The Au-Ag nanoparticles were found to have a wide spectrum of applications in various fields including ultraraf colorimetric detection of H2O2, photothermal therapy, and antibiotic therapy. Moreover, these nanoparticles were found to have effective anti-bacterial properties without any harmful cytotoxic side effects, guaranteeing the bright future of Ag and Au in the biomedical field (Jiang et al., 2019).

Another interesting study conducted by Lateef et al. (2016), used Cola nitida, commonly referred to as Kolanut for the biosynthesis of Ag-Au nanoparticles. The antifungal study of these nanoparticles revealed that they were efficacious against Aspergillus flavus, Aspergillus fumigatus, and Aspergillus niger. The Kolanut nanoparticles were also studied for their larvicidal properties and exhibited a mortality rate of 80-100% within 24 hours, when exposed to the Anopheles mosquito larva. These nanoparticles also showed blood anticoagulation properties as well as thrombolytic potential making them suitable for
nanostructures were synthesized using metal nanoparticles, Ag, Au and Ag-Au bimetallic nanoparticles were synthesis using the aqueous leaf extract of Moringa oleifera. The results exhibited the effectiveness of the Ag-Au nanoparticles against the Hep2 cell line (Gupta et al., 2020). Bimetallic nanoparticles are making their way into the diagnostic and therapeutic sciences, thus making them extremely ideal theranostic agents and are also being studied for various biomedical applications that include drug delivery, antimicrobial property, antifungal nature, larvicidal nature amongst others.

**TABLE I: ANTICANCER ACTIVITIES OF BIOSYNTHESIZED SILVER NANOPARTICLES**

| Study          | Plant Species                  | Size of Nanoparticles | Properties exhibited                                                                 |
|---------------|--------------------------------|-----------------------|-------------------------------------------------------------------------------------|
| Maria et al., 2014 | Zizyphus xylopyrus               | 60-70 nm              | Antimicrobial, Drug delivery system                                                  |
| Mukherjee et al., 2014 | Olax scandens                  | 30-60 nm              | Antibacterial, Anti-cancer on human lung cancer, breast cancer, and mouse melanoma, Drug delivery vehicle, Imaging facilitator |
| Anna et al., 2018 | Punica granatum                | 6-45 nm               | Anti-cancer towards human lung cancer, Antimicrobial, Antioxidant                    |
| Kadam et al., 2020 | Brassica oleracea var. botrytis | 35.08 nm              | Photocatalytic activity for degrading MB dye, Biosensing of Hg²⁺ ion                |
| Vedelego et al., 2018 | Seriphidium quettense          | 120.4 nm              | Antibacterial, Anti-cancer on human liver cancer, Antifungal, High biocompatibility towards healthy erythrocytes |
| Gurunathan et al., 2013 | Gnanoderma neo-                              | 5 nm                  | Cytotoxic effects, Apoptotic features on human breast cancer                          |
| Ahmed et al., 2019 | Jurinea dolomiaeae             | -                     | Anti-cancer towards human cervical cancer and human breast cancer, Antioxidant potential |
| Ullah et al., 2020 | Fagonia indica                 | 10-60 nm              | Anti-cancer on human breast cancer, Antioxidant, Apoptotic features, Activating caspase 3 and 9 |
| Atale et al., 2017 | Syzygium cumini               | 40-100 nm             | Anti-diabetic, Cardioprotective potential                                            |
| Aziz et al., 2019 | Piniferomsora indica          | 6-15 nm               | Anti-cancer on human breast cancer, liver cancer, and cervical cancer                 |
| Balakumaran et al., 2015 | Guignardia mangiferae          | 5-30 nm               | Antimicrobial, Anti-cancer activity on human cervical cancer and breast cancer         |
| Venugopal et al., 2017 | Beta vulgaris                  | 5-20 nm               | Anti-cancer effect on human breast cancer, lung cancer, and pharynx cancer           |
| Kuppusamy et al., 2016 | Commelina nadiflora           | 24-150 nm             | Anti-cancer effect on human colon cancer                                            |
| Dinparvar et al., 2020 | Cuminum cyminum              | -                     | Anti-cancer on human breast cancer                                                  |
| Jeyaraj et al., 2013 | Sesbania grandiflora           | 22 nm                 | Anti-cancer on human breast cancer                                                  |
| Ramar et al., 2015 | Solanum trilobatum            | 41.90 nm              | Antibacterial, Anticancer on human breast cancer                                    |
| Sharma et al., 2015 | Rheum emodi                   | 27.50 nm              | Anti-cancer on human breast cancer                                                  |
| Heydari et al., 2015 | Oak fruit hull                | 40 nm                 | Anti-cancer on human breast cancer                                                  |
| Kathiravan et al., 2014 | Melia dubia                   | 7.3 nm                | Anti-cancer on human breast cancer                                                  |
| Reddy et al., 2013 | Piper longum                  | 46 nm                 | Antimicrobial, Anti-cancer on human breast cancer, Antioxidant                       |
| Shawkey et al., 2011 | Citrullus colocynthis         | 7-19 nm               | Anti-cancer effect on human breast cancer, liver cancer, colon cancer, and intestinal cancer |
| Venugopal et al., 2017 | Syzygium aromaticum           | 5-20 nm               | Anti-cancer on human breast cancer and lung cancer                                  |
| Bhattacharya et al., 2008 | Gelsemium sempervirens        | -                     | Anti-cancer effect on human cervical cancer                                          |
| Venkatesan et al., 2014 | Rosa damascena               | 84 nm                 | Anti-cancer potential towards human lung adenocarcinoma, Biocompatibility (selectively destroys cancer cells, while leaving healthy cells untouched) |
| Khanra et al., 2015 | Scoparia dulcis              | 15-25 nm              | Antibacterial, anti-cancer effect on human lung cancer                               |
| Palaniappan et al., 2014 | Cymodocea serrulata          | 29.28 nm              | Anti-cancer effect on human lung cancer                                              |
| Khanra et al., 2015 | Croton bonplandianum          | 15-40 nm              | Antibacterial, anti-cancer effect on human ovarian cancer and human lung cancer       |
| AlSalhi et al., 2016 | Pimpinella anisum             | 3.2-16 nm             | Antimicrobial, Anti-cancer property towards human neonatal skin cancer and human colon cancer |
| Manikandan et al., 2015 | Rosa indica                  | 23.52-60.83 nm        | Anti-bacterial, Anti-cancer effect on human colon adenocarcinoma, Anti-inflammatory |
| Prabhu et al., 2013 | Vitex negundo                 | 22 nm                 | Anti-cancer effect on human colon cancer, Apoptotic features                         |
| Arunachalam et al., 2014 | Gymnema sylvestre             | 22 nm                 | Anti-cancer property towards human colon adenocarcinoma, Anti-ulcerative             |
| Jeyaraj et al., 2012 | Podophyllum hexandrum         | 14 nm                 | Anti-cancer on human cervical cancer                                                |
| Vijistella et al., 2014 | Heliotropium indicum          | 80-90 nm              | Anti-cancer effect on human cervical cancer, antimicrobial potency                   |
| Sukirtha et al., 2011 | Melia azedarach              | 78 nm                 | Anti-cancer property on human cervical cancer and mouse lymphoma                    |
| Misra et al., 2012 | Azadirachta indica           | 2-18 nm               | Anti-cancer on human cervical cancer                                                |
| Devi et al., 2012 | Ulva lactuca                 | 20-56 nm              | Anti-cancer towards human breast cancer, cervical cancer, and intestinal cancer       |
| Satyavani et al., 2011 | Citrullus colocynthis        | 31 nm                 | Anti-cancer towards human larynx cancer                                              |
| Satyavani et al., 2012 | Stoeleda monoica             | 31 nm                 | Anti-cancer towards human larynx cancer                                              |
| Kumar et al., 2015 | Andean mora                  | 12-50 nm              | Antioxidant, Drug delivery on Hep-G2 cell line cancer cells                          |
| Pandian et al., 2015 | Allium sativum               | 100-800 nm            | Anti-cancer                                                                          |
| He et al., 2013 | Chrysanthemum moriolium      | 20-50 nm              | Antimicrobial                                                                         |
| Longhi et al., 2016 | Fusarium oxysporum           | -                     | Antifungal, Biofilm inhibition in *Candida albicans*                                  |
| Gupta et al., 2020 | Moringa oleifera             | 11-25 nm              | Anti-cancer on human breast cancer, human liver cancer                               |
| Sivamanathithi et al., 2019 | Terminalia chebula           | 20 nm                 | Anticoagulant, Thrombolytic, Larvicidal, Antifungal, Catalytic potential             |
| Lateet al., 2016 | Cola nitida                 | 17-91 nm              | Anti-cancer                                                                          |
VII. CONCLUSION

With the demand for nanotechnology and nanomedicine increasing exponentially in the global market, green silver nanoparticles prove to be the perfect theranostic agents. Their lack of toxicity and high biocompatibility make them suitable therapeutic agents of malignant ailments like cancer. Their properties of fluorescence and imaging pave the way for a new era of eco-friendly diagnostic techniques. Even though there are many barriers to the commercial production and use of b-AgNPs, they have a bright future in nanobiotechnology and cancer theranostics. However, the bioavailability, toxicity, compatibility, drug delivery, and body clearance of these b-AgNPs need to be studied thoroughly to evolve and expand their scope in biomedical applications.

CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest.

REFERENCES

Abel, B., Cookson, S., Mohamed, M., Williams, R., Unalan, H. E., & Aslan, K. (2015). Metal-enhanced fluorescence from silver nanowires with high aspect ratio on glass slides for biosensing applications. The Journal of Physical Chemistry C, 119(1), 675-684.

Ahmad, M. Z., Akhter, S., Jain, G. K., Rahman, M., Pathan, S. A., Ahmad, F. J., & Khar, R. K. (2010). Metallic nanoparticles: technology overview & drug delivery applications in oncology. Expert opinion on drug delivery, 7(8), 927-942.

Ahmed, M. J., Murtaza, G., Rashid, F., & Iqbal, J. (2019). Eco-friendly green synthesis of silver nanoparticles and their potential applications as antioxidant and anticancer agents. Drug development and industrial pharmacy, 45(10), 1682-1694.

Ahmed, N., Fessi, H., & Elaiassri, A. (2012). Theranostic applications of nanoparticles in cancer. Drug discovery today, 17(17-18), 928-934.

AlSalhi, M. S., Devanesan, S., Alfuraydi, A. A., Vishnubalalji, R., Munusamy, M. A., Murugan, K., … & Benelli, G. (2016). Green synthesis of silver nanoparticles using Pimpinella anisum seeds: antimicrobial activity and cytotoxicity on human neonatal skin stromal cells and colon cancer cells. International Journal of nanomedicine, 11, 4439.

Annu, M., Ahmed, S., Kaur, G., Sharma, P., Singh, S., & Iram, S. (2018). Evaluation of the antioxidant, antibacterial and anticancer (lung cancer cell line A549) activity of Puncata granatum mediated silver nanoparticles. Toxicology research, 7(5), 923-930.

Arunachalam, K. D., Arun, L. B., Annamalai, S. K., & Arunachalam, A. M. (2012). Anticancer activity of an alkaloid of the plant Gelsemium sempervirens. Experimental Biology and Medicine, 237(12), 1591-1601.

Bilal, M., Rasheed, T., Iqbal, H. M., Li, C., Hu, H., & Zhang, X. (2017). Development of silver nanoparticles loaded chitosan-alginic constructs with biomedical potentials. International journal of biological macromolecules, 105, 393-400.

Brown, A. N., Smith, K., Samuels, T. A., Li, J., Obare, S. O., & Scott, M. E. (2012). Nanoparticles functionalized with ampicillin destroy multiple-antibiotic-resistant isolates of Pseudomonas aeruginosa and Enterobacter aerogenes and methicillin-resistant Staphylococcus aureus. Applied and environmental microbiology, 78(8), 2768-2774.

Cassano, D., Mapano, A. K., Summa, M., Vlaminid, Y., Giannone, G., Santi, M., … & Voliani, V. (2019). Biosafety and biokinetics of noble metals: The impact of their chemical nature. ACS Applied Bio Materials, 2(10), 4464-4470.

Cherkulka, K., Manickavasagam Lekshmi, K., Uthaman, S., Cho, K., Cho, C. S., & Park, I. K. (2016). Multifunctional inorganic nanoparticles: Recent progress in thermal therapy and imaging. Nanomaterials, 6(4), 76.

Devi, J. S., & Bhimba, B. V. (2012). Anticancer Activity of Silver Nanoparticles Synthesized by the Seaweed Ulva lactuca Invitro 1.2. doi: 10.4172/scientificreports. 242 Page 2 of 5 Volume 1• Issue 4• 2012 silver nitrate solution was added to the filtrate slowly under magnetic stirring conditions for even coating of silver and subjected to heating at 12 C for 10 min. The extract is used as reducing and stabilizing agent for AgNPs. This one pot green synthesis was the modified method followed by Vigneshwaran et al.[18].

Dinparvar, S., Bagirova, M., Allahverdiev, A. M., Abamor, E. S., Safarov, T., Aydogdu, M., & Aktas, D. (2020). A nanotechnology-based new approach in the treatment of breast cancer: Biosynthesized silver nanoparticles using Cuminum cyminum L. seed extract. Journal of Photochemistry and Photobiology B: Biology, 208, 111902.

Duran, N., & Seabra, A. B. (2018). Biogenic synthesized Ag/Ag nanoparticles: production, characterization, and applications. Current Nanoscience, 14(2), 82-91.

El-Sheekh, M., M., Shaban, M. T., Hassan, L., & Morsi, H. H. (2020). Antiviral activity of algae biosynthesized silver and gold nanoparticles against Herps Simplex (HSV-1) virus in vitro using cell-line culture technique. International Journal of Environmental Health Research, 1-12.

Fridman, J. S., & Low, S. W. (2003). Control of apoptosis by p53. Oncogene, 22(56), 9030-9040.

Gajbiye, S., & Sakharwade, S. (2016). Silver nanoparticles in cosmetics. Journal of Cosmetics, Dermatological Sciences and Applications, 6(1), 48-53.

Gardner-Torrey, J. L., Gomez, E., Peralta-Videa, J. R., Parsons, J. G., Troiani, H., & Jose-Yacaman, M. (2003). Algal sprouts: a natural source for the synthesis of silver nanoparticles. Langmuir, 19(4), 1357-1361.

Gupta, S., Hemlata, H., & Tejavath, K. (2020). Synthesis, characterization and comparative anticancer potential of phytosynthesized mono and bimetallic nanoparticles using Moringa oleifera leaf extract. Beilstein Arch, 1, 95.

Gurunathan, S., Han, J. W., Eppakayala, V., Jeyaraj, M., & Kim, J. H. (2013). Cytotoxicity of biologically synthesized silver nanoparticles in MDA-MB-231 human breast cancer cells. BioMed research international, 2013.

Gurunathan, S., Raman, J., Abd Malek, S. N., John, P. A., & Vikineswary, S. (2013). Green synthesis of silver nanoparticles using Ganoderma neo-japonicum Imazeki: a potential cytotoxic agent against breast cancer cells. International journal of nanomedicine, 8, 4399.

He, Y., Du, Z., Liu, H., Xua, Q., Tang, Z., Zheng, X., … & Zhao, F. (2013). Green synthesis of silver nanoparticles by Chrysanthemum morifolium Ramat. extract and their application in clinical ultrasound gel. International Journal of Nanomedicine, 8, 1809.

Heydari, R., & Rashidipour, M. (2015). Green synthesis of silver nanoparticles using extract of oak fruit hull (Jaff): synthesis and in vitro cytotoxic effect on MCF-7 cells. International journal of breast cancer, 2015.

Irivani, S., Korbekandi, H., Mirmohammadi, S. V., & Zolfaghari, B. (2014). Synthesis of silver nanoparticles: chemical, physical and biological methods. Research in pharmaceutical sciences, 9(6), 385.
Jahangirian, H., Lernrasri, E. G., Webster, T. J., Rafiee-Moghaddam, R., & Abdollahi, Y. (2017). A review of drug delivery systems based on nanotechnology for green chemical and green nanomedicine. *International journal of nanomedicine*, 12, 2957.

Jee, L., Reddy, R. J., Marahewan, T., Asokan, G. S., Dany, A., & Anand, B. (2014). Theranostics: A treasured tailor for tomorrow. *Journal of pharmacy & biomedicined sciences*, (Suppl 1), S6.

Jenal, A., Thomas, S., Perry, T., & Thomas, 2002. Cancer statistics, 2002. *Ca-A Cancer Journal for Clinicians*, 52(1), 23-47.

Jeyaraj, M., Rajesh, M., Arun, R., MubarakAli, D., Sathishkumar, G., Sivanandhan, G., ... & Ganapathi, A. (2013). An insight on plant green synthesized nanoparticles as new tools in cancer therapy: molecular imaging with theranostic nanoparticles. *Green Chemistry Letters and Reviews*, (54), 3-12.

Jeyaraj, M., Sathishkumar, G., Sivanandhan, G., MubarakAli, D., Rajesh, M., Arun, R., ... & Ganapathi, A. (2013). Biogenic silver nanoparticles for cancer treatment: an experimental report. *Colloids and Surfaces B: Biointerfaces*, 102, 708-717.

Jeyaraj, M., Sathishkumar, G., Sivanandhan, G., MubarakAli, D., Rajesh, M., Arun, R., ... & Ganapathi, A. (2013). Biogenic silver nanoparticles for cancer treatment: an experimental report. *Colloids and Surfaces B: Biointerfaces*, 102, 708-717.

Kang, H., Mintri, S., Menon, A. V., Lee, H. Y., Choi, H. S., & Kim, J. (2015). Pharmacokinetics, pharmacodynamics and toxicology of theranostic nanoparticles. *Nanoscale*, 7(45), 18884-18882.

Karmous, I., Pandey, A., Haj, K. B., & Chauoi, A. (2020). Efficiency of the green synthesized nanoparticles as new tools in cancer therapy: insights on plant-based engineered nanoparticles, biophysical properties, and anticancer roles. *Biological Trace Element Research*, 196(1), 330-342.

Kathiravan, Ravi, S., & Ashokkumar, S. (2014). Synthesis of silver nanoparticles from Melia dubia leaf extract and their in vitro anticancer activity. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 130, 116-121.

Kelly, F. M., & Johnston, J. H. (2011). Colored and functional silver nanoparticle–wool fiber composites. *ACS applied materials & interfaces*, 4(4), 1083-1092.

Khanra, K., Panja, S., Choudhuri, I., Chakraborty, A., & Bhattacharyya, N. (2015). Evaluation of antibacterial activity and cytotoxicity of green synthesized silver nanoparticles using Scoparia dulcis. *Nano Biomed. Eng.*, 7(3), 128-133.

Khanra, K., Panja, S., Choudhuri, I., Chakraborty, A., & Bhattacharyya, N. (2015). Antibacterial activity and cytotoxicity effect of silver nanoparticle synthesized from Croton longimanum Bauil. leaves. *Nanomedicine Journal*, 3(1), 15-22.

Kikuchi, M., Kuroki, S., Kayama, M., Sakaguchi, S., Lee, K. K., & Yonehara, S. (2012). Protease activity of procaspase-8 is essential for cell survival signals involving both apoptosis and nonapoptotic cell death dependent on receptor-interacting protein kinase 1 (RIP1) and RIP3. *Journal of Biological Chemistry*, 287(49), 41165-41173.

Kumar, B., Smita, K., Seqqat, R., Benalcazar, K., Grijalva, M., & Cumbal, L. (2016). In vitro evaluation of silver nanoparticles cytotoxicity on Hepatic cancer (Hep-G2) cell line and their antioxidant activity. *Green approach for fabrication and application. Journal of Photochemistry and Photobiology B: Biology*, 159, 8-13.

Kuppasamy, P., Ishwan, S. W., Al-Zikri, P. N. H., Suriyah, W. H., Soundharrajan, I., Govindan, N., ... & Yusoff, M. (2016). In vitro anticancer activity of Ag nanoparticles synthesized using Commelina communis L. aqueous extract against HCT-116 colon cancer cells. *Biological trace element research*, 173(2), 297-305.

Lam, P. L., Wong, W. Y., Bian, Z., Chui, C. H., & Gamba, R. (2017). Recent advances in green nanoparticle systems for drug delivery: efficient delivery and safety concern. *Nanomedicine*, 12(4), 357-385.

Lateef, A. Oju, S. A., Folani, B. I., Guegum-Kana, E. B., Beukes, L. S. (2016). Kolanut (Cola nitida) mediated synthesis of silver–gold alloy nanoparticles: antifungal, catalytic, larvicidal and thrombolytic applications. *Journal of Cluster Science*, 27(5), 1561-1577.

Li, D. G., Zhu, G., Li, S., Yang, W., Fu, W., Li, K., & Wang, X. (2015). Synthesis of silver nanoparticles using medicinal Zizyphus xylopyrus cork extract. *Applied nanoscience*, 5(6), 755-762.

Liu, N., Zhang, Y., Chen, Y., Guo, X., Ding, W., Ali, S. F., ... & Chen, T. (2012). Silver nanoparticle-induced mutations and oxidative stress in mouse lymphoma cells. *Environmental and molecular mutagenesis*, 53(6), 409-419.

Mishra, A., Mehl, S. J., Irshad, M., Ali, A., Sardar, M., Moshahid, M., & Rizvi, A. (2012). Effect of biologically synthesized silver nanoparticles on human cancer cells. *Science of Advanced Materials*, 4(12), 1200-1206.

Mukherjee, S., & Patra, C. R. (2017). Biologically synthesized metal nanoparticles: recent advancement and future perspectives in cancer theranostics. *European Journal of Pharmacology and Biotechnology*, 120, 23-38.

Pandian, A. M. K., Karthikeyan, C., Rajasimman, M., & Dinesh, M. G. (2015). Synthesis of silver nanoparticle and its application. *Ecotoxicology and environmental safety*, 121, 211-217.

Parvez, K., Banse, V., & Ledwani, I. (2016). Green synthesis of nanoparticles: their advantages and disadvantages. In *AIP conference proceedings* (Vol. 1724, No. 1, p. 020048). AIP Publishing LLC.

Patra, S., Mukherjee, S., Barui, A. K., Ganguly, A., Sreedhar, B., & Patra, C. R. (2015). Green synthesis, characterization of gold and silver nanoparticles and their potential application in cancer therapeutic agents. *Materials Science and Engineering: C*, 53, 298-309.

Prabhu, D., Arulvasu, C., Babu, G., Manikandan, R., & Srinivasan, P. (2013). Biologically synthesized green silver nanoparticles from leaf extract of Vitex negundo L induce growth-inhibitory effect on human cervical cancer cells. *Appl. Biochem. Biotechnol.*, 171(6), 2093-2104.

Qaisar, S., Zohra, T., Khalil, A. T., Saqib, S., Ayaz, M., Ahmad, A., & Shinwari, Z. K. (2019). Serum heparin quenched mediated green synthesis of biogenic silver nanoparticles and their theranostic applications. *Chem. Eng. J.*, 379, 257-267.

Rama, M., Manikandan, B., Marinuthu, P. N., Raman, T., Mahalingam, A., Subramaniam, P., ... & Munusamy, A. (2015). Synthesis of silver nanoparticles using Solanum trilobatum fruits extract and its antibacterial, cytotoxic activity against human breast cancer cell line MCF 7. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 140, 223-228.

Rauwel, P., Kütünlü, S., Ferdo, S., & Rauwel, E. (2015). A review on the green synthesis of silver nanoparticles and their morphologies studied via TEM. *Advances in Materials Science and Engineering*, 2015.

Reddy, J. N., Vali, D. N., Rani, M., & Rani, S. S. (2014). Evaluation of antioxidant, antibacterial and cytotoxic effects of green synthesized silver nanoparticles by Piper longum fruit. *Materials Science and Engineering: C*, 34, 115-122.

Sahoo, A. K., Goswami, U., Dutta, D., Banerjee, S., Chattopadhyay, A., & Ghosh, S. S. (2016). Silver nanocluster embedded composite nanoparticles for targeted prodrug delivery in cancer theranostics. *ACS Biomaterials Science & Engineering*, 12(1), 140-149.

Salvadori, M. R., Ando, A. R., & Corrêa, B. (2022). Bio-separator and bio-synthesizer of metallic nanoparticles-A new vision in bioremediation. *Materials Letters*, 306, 130878.

Satyavani, K., Gurudeeban, S., Ramanathan, T., & Balasubramanian, T. (2011). Biomedical potential of silver nanoparticles synthesized from
calli cells of Citrullus colocynthis (L.) Schrad. Journal of nanobiotechnology, 9(1), 1-8.

Satyavani, K., Gurudeeban, S., Ramanathan, T., & Balasubramanian, T. (2012). Toxicity study of silver nanoparticles synthesized from Suaeda monoica on Hep-2 cell line. Avicenna journal of medical biotechnology, 4(1), 35.

Sharma, D., Ledwani, L., & Bhatnagar, N. (2015). Antimicrobial and cytotoxic potential of silver nanoparticles synthesized using Rheum emodi roots extract. New Frontiers in Chemistry, 24(2), 121.

Shawkey, A. M., Rabeh, M. A., Abdullall, A. K., & Abdellatif, A. O. (2013). Green nanotechnology: anticancer activity of silver nanoparticles using Citrullus colocynthis aqueous extracts. Adv. Life Sci. Technol. 13, 69-70.

Sivamaruthi, B. S., Ramkumar, V. S., Archunan, G., Chaiyasut, C., & Suganthy, N. (2019). Biogenic synthesis of silver palladium bimetallic nanoparticles from fruit extract of Terminalia chebula-In vitro evaluation of anticancer and antimicrobial activity. Journal of Drug Delivery Science and Technology, 51, 139-151.

Sriniv, P., Chen, Y. T., Vittur, V., Marquez, M. D., & Lee, T. R. (2018). Bimetallic nanoparticles: enhanced magnetic and optical properties for emerging biological applications. Applied Sciences, 8(7), 1106.

Sukirtha, R., Priyanka, K. M., Antony, J. J., Kamalakkannan, S., Thangam, R., Gunasekaran, P., ... & Achiran, S. (2012). Cytotoxic effect of Green synthesized silver nanoparticles using Melia azedarach against in vitro HeLa cell lines and lymphoma mice model. Process Biochemistry, 47(2), 273-279.

Tiquia-Arashiro, S., & Rodrigues, D. (2016). Application of nanoparticles. In Extremophiles: Applications in Nanotechnology (pp. 163-193). Springer, Cham.

Ullah, I., Khalil, A. T., Ali, M., Iqbal, J., Ali, W., Alarifi, S., & Shinwari, Z. K. (2020). Green-synthesized silver nanoparticles induced apoptotic cell death in MCF-7 breast cancer cells by generating reactive oxygen species and activating caspase 3 and 9 enzyme activities. Oxidative medicine and cellular longevity, 2020.

Vanaja, M., Paulkumar, K., Baburaja, M., Rajeshkumar, S., Gnanajothi, G., Malarkodi, C., ... & Annadurai, G. (2014). Degradation of methylene blue using biologically synthesized silver nanoparticles. Bioinorganic chemistry and applications, 2014.

Varghese Alex, K., Tamil Pavai, P., Rugmini, R., Shiva Prasad, M., Kamarthi, K., & Sekhar, K. C. (2020). Green synthesized Ag nanoparticles for bio-sensing and Photocatalytic applications. ACS omega, 5(22), 13123-13129.

Vedelago, J., Gomez, C. G., Valente, M., & Mattea, F. (2018). Green synthesis of silver nanoparticles aimed at improving theranostics. Radiation Physics and Chemistry, 146, 55-67.

Venkatesan, B., Subramanian, V., Tumala, A., & Vellaichamy, E. (2014). Rapid synthesis of biocompatible silver nanoparticles using aqueous extract of Rosa damascena petals and evaluation of their anticancer activity. Asian Pacific journal of tropical medicine, 7, S294-S300.

Venugopal, K., Ahmed, H., Manikanandan, E., Arul, K. T., Kavitha, K., Moodley, M. K., ... & Bhaskar, M. (2017). 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. Journal of Photochemistry and Photobiology B: Biology, 173, 99-107.

Venugopal, K., Rather, H. A., Rajagopal, K., Shanithi, M. P., Sherriff, K., Iliyias, M., ... & Maoza, M. (2017). Synthesis of silver nanoparticles (Ag NPs) for anticancer activities (MCF 7 breast and A549 lung cell lines) of the crude extract of Syzygium aromaticum. Journal of Photochemistry and Photobiology B: Biology, 167, 282-289.

Vijistella Bai, G. (2014). Green Synthesis Of Silver Nanostructures Against Human Cancer Cell Lines And Certain Pathogens. International Journal of Pharmaceutical, Chemical & Biological Sciences, 4(1).

Xie, Z., Su, Y., Kim, G. B., Selvi, E., Ma, C., Aragon-Sanabria, V., ... & Yang, J. (2017). Immune Cell-Mediated Biodegradable Theranostic Nanoparticles for Melanoma Targeting and Drug Delivery. Small, 13(10), 1603121.

Yetisgin, A. A., Cetinel, S., Zuvin, M., Kosar, A., & Kutlu, O. (2020). Therapeutic nanoparticles and their targeted delivery applications. Molecules, 25(9), 2193.

Yhee, J. Y., Son, S., Kim, N., Choi, K., & Kwon, I. C. (2014). Theranostic applications of organic nanoparticles for cancer treatment. Mrs Bulletin, 39(3), 239-249.

Zavaleta, C., Ho, D., & Chung, E. J. (2018). Theranostic nanoparticles for tracking and monitoring disease state. SLS TECHNOLOGY: Translating Life Sciences Innovation, 23(3), 283-293.

Zeelan, G. S. G., EL-Razik, K. A. A., Allam, A. M., Abdalhamed, A. M., & Zeina, H. A. A. (2020). Evaluations of potential antiviral effects of green zinc oxide and silver nanoparticles against bovine herpesvirus-1. Adv. Anim. Vet. Sci, 8(4), 433-443.