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Green Synthesized Silver Nanoparticles of *Portulaca oleracea* and its Anticancer Activity Against MCF-7 Cell Line

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Abstract: Nanotechnology has been a burning field for the researchers in recent years. Nanotechnology deals with the Nanoparticles having a size of 1-100 nm in one dimension which is used significantly in medicinal chemistry, atomic physics, and all other known fields. Nanoparticles are used immensely due to its small size, orientation, physical properties, which are reportedly shown to change the performance of any other material which is in contact with these tiny particles. These particles can be prepared easily by different chemical, physical, and biological approaches. But the biological approach is the most emerging approach for preparation, because, this method is easier than the other methods, ecofriendly and less time consuming. The Green synthesis was done by using the aqueous solution of *Portulaca oleracea* leaf extract and AgNO₃. Silver was of a particular interest for this process due to its evocative physical and chemical properties. A fixed ratio of plant extract to metal ion was prepared and the color change was observed which proved the formation of nanoparticles. The nanoparticles were characterized by UV-vis Spectrophotometer, FTIR, SEM and TEM. The nanoparticles were found have the size ranges from 50- 120 nm. In the present study we synthesize the nanoparticles using *Portulaca oleracea* and analyzed the synthesized nanoparticles for its anticancer properties.

Keywords: *Portulaca oleracea*, Nanotechnology, Nanoparticles, Green Synthesis

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Introduction

Due to increased industrialization and urbanization, our environment is being loaded with large amount of perilous and superfluous chemical, gases or substances. It is our need to learn about the secrets that are present in the nature and its products which leads to the growth of advancements in the synthesis processes of nanoparticles. Nanotechnology applications are highly suitable for biological molecules, because of

their exclusive properties. The biological molecules undergo highly controlled assembly for making them suitable for the metal nanoparticle synthesis which was found to be reliable and ecofriendly (Li *et al.*, 2007).

In recent time, noble metal nanoparticles have been the area of focus of researches as they show improved physical and chemical properties in the fields of electronic, optical, mechanical, magnetic

and chemical processing (Srikar *et al.*, 2016). This could be due to their small size with large specific surface, quantum size effect and electronic structures. Hence, metallic nanoparticles have found relevance in the fields of electronics, photochemical, biomedicine, chemistry, catalysis and photonics (Rafique *et al.*, 2017).

About 70 % of elements in the periodic table are considered as metals, 5 are metalloids with both metallic and non-metallic properties. A metal is any material with high conductivity and reflectivity that can be deformed plastically. They are usually malleable, ductile and shiny. Different characteristics are displayed by metals when exposed to many conditions (Awwad *et al.*, 2012). Metals such as nickel, vanadium, molybdenum, cobalt and the platinum group metals enable the catalytic reactions for the synthesis of many organic chemicals from petroleum. Gold, silver and platinum (noble metals) have found applications in biomedical-drug delivery, imaging, photothermal therapy and immunochromatography (Roy *et al.*, 2019).

In recent days, silver nanoparticles have been synthesized from the naturally occurring sources like green tea (*Camellia sinensis*), Neem (*Azadirachta indica*), leguminous shrub (*Sesbania drummondii*), various leaf broth, natural rubber, starch, Aloe vera plant extract, lemongrass leaves extract, etc. (Okafor *et al.*, 2013). With respect to the microbes, the silver nanoparticles get attached to the cell wall, thereby disturbing the permeability of cell wall and cellular respiration. The nanoparticles may also penetrate deep inside the cell wall, thus causing cellular damage by interacting with phosphorus and sulfur containing compounds, such as DNA and protein present inside the cell (Mallikarjuna *et al.*, 2011).

In the developed world, breast cancer is one of the most common types of cancers among women, which accounts for 15–20% of all cancer deaths in the mid-1990s (Noori and Hassan, 2012). Breast cancer is the most commonly occurring cancer in women, comprising almost one-third of all

malignancies (Zhao *et al.*, 2009). It accounts for approximately 25% of all female malignancies with a higher prevalence in developed countries. Breast cancer is the second leading cause of cancer-related death among females in the world (Liu *et al.*, 2013).

Development of breast cancer cell targeting drug without affecting the normal cells is a challenging task in the field of cancer drug discovery. There is limited success of clinical therapies including radiation, chemotherapy, immunomodulation and surgery in treating cancer, in addition to high morbidity and mortality rates and severe side effects such as bone marrow depression, leucopenia, anaemia, alopecia, hyperuricaemia, teratogenicity, carcinogenicity, reduces spermatogenesis in men and Amenorrhea in women. Thus, there is an imperative need of new cancer management. Herbal plants have long been shown to be excellent and reliable sources for the development of novel anti-cancer drugs (Ramya *et al.*, 2017).

A wide variety of anti-cancer agents have been described for the treatment of various kinds of cancers, however, they face the major failure attributed to the development of multi-drug resistance (MDR). Thus, it is a major challenge to the pharmaceutical industry, since the development of MDR limits the use of anticancer agents in chemotherapy such as vinca alkaloids (vincristine, vinblastine), taxanes (paclitaxel), epipodophyllotoxins (etoposide, teniposide) and the topoisomerase inhibitors (topotecan) (Thomas and Coley, 2003). Therefore, the development of potent and safe anti-cancer agents, which are devoid of MDR or simultaneously modifies the MDR, is a crucial aspect of modern cancer research. The wide biological potential of naturally occurring lead molecules have always been considered as promising aspect, and researchers have indulged in the development of these molecular libraries (Koehn and Carter, 2005). In 1948, an acridone alkaloid, acronycine (Bach *et al.*, 2007) was isolated from *Acronychia*

baueri plant belonging to Rutaceae family, displayed a broad spectrum activity against a variety of experimental tumor models including sarcoma, myeloma, carcinoma, and melanoma (Nguyen *et al.*, 2009).

A successful anticancer drug should kill or immobilize the cancer cells without causing excessive damage to the normal cells. This idea is difficult, or perhaps impossible, to attain and that is why cancer patients frequently suffer unpleasant side effects when undergoing treatment. Synthesis of modifications of known drug continues as an important aspect of research. There is a continued need for new prototype-new templates to use in the design of potential chemotherapeutic agents; natural products are providing such templates. Recent studies of tumor-inhibiting compound of plant origin have yielded an impressive array of novel structures. Many of these structures are extremely complex, and it is most unlikely that such compounds would have been synthesized in empirical approaches to new drugs. Modern medicine attributes most cases of cancer to changes in DNA that reduce or eliminate the normal controls over cellular growth, maturation, and programmed cell death (Jaikumar and Jasmine, 2016).

Chemotherapy and radiotherapy are main treatments for cancer at present. However, chemotherapy and radiotherapy are generally expensive for patients. Additionally, chemotherapy leads to drug resistance and severe side effects for patients. Therefore, it is necessary to develop highly efficient natural chemotherapeutic agents with low cost, low drug resistance and less side effects (Nguyen *et al.*, 2009).

P. oleracea are used in treatments for internal parasites. The plant always is mixed with other plants (*Chenopodium ambrosioides*). *P. oleracea* is listed as a treatment for parasites, a blood-cleanser, and to refresh the digestive system. The leaves and tops are used as anti-hemorrhagic poultices. The roasted seeds are considered

diuretic and antidysentery. The seeds are also used in applications for burns and scalds. In general, their medicinal uses are similar to those mentioned for the herb. Chemical constituents such as steroids, vitamins, minerals, fatty acids, alkaloids, saponins, etc. have been isolated from the plant.

In the present study we synthesize the nanoparticles using *Portulaca oleracea* and analyzed the synthesized nanoparticles for its anticancer properties.

Materials and Methods

Plant collection and extraction:

The fresh disease free *Portulaca oleracea* plants (Fig. 1) were collected from Chennai district, Tamil Nadu, India. The leaves were dried under shade at room temperature for about 14 days and powdered and sieved to get a fine powder using an electric blender. The aqueous extract was prepared using distilled water. The powder was soaked in distilled water for 1 day and filtered and dried, then stored it under 4 C for further use.

Fig. 1: *Portulaca oleracea*.

Qualitative phytochemical analysis:

The prepared aqueous extract was subjected to qualitative phytochemical analysis for the presence of phytocompounds using standard procedures outlined by Harbourne (1998).

Synthesis of AgNPs using aqueous extract:

1 ml of the aqueous extract (10 mg/100 ml) was mixed with 9 ml of 1 mM silver nitrate solution and the mixtures were incubated in a dark room at

27 C and change in colour was noted. Immediately the colour changed from light yellow to dark brown within 25 min (Fig. 2). The synthesized AgNPs were taken further to characterize its nanoparticle nature and also to assess its antioxidant and anticancer potential against MCF-7 cell line.

Fig. 2: AgNPs synthesis and colour change from yellow to dark brown colour of *P. oleracea*.

UV-Vis Spectral Analysis:

UV-Vis spectrophotometer (Shimadzu, Japan) was used to characterize the aqueous and aqueous based synthesized AgNPs at a resolution of 1 nm

between 200 to 800 nm.

FT-IR Spectral Analysis:

FT-IR spectroscopy studies were carried out for aqueous and aqueous based AgNPs. One mg of the sample was scanned in the region of range 4000-500 cm^{-1} using Alpha II- Bruker- Opus.

High Resolution-Transmission Electron Microscopy (HR-TEM):

The high resolution transmission electron microscopy (HR-TEM) images of synthesized AgNPs were obtained using JEOL model 3010 instrument operated at 200 kV and a beam current of 104.1 μA . Sample for this analysis was prepared by coating the aqueous AgNPs on carbon coated copper grids (300 mesh size) by slow evaporation and then allowed to dry in vacuum at 25 C for overnight.

Antioxidant Assays:

1,1-diphenyl-2-picrylhydrazine (DPPH) radical scavenging assay (Blois, 1958):

DPPH is a stable free radical that reacts with compounds that can donate a hydrogen atom. This method is based on the scavenging of DPPH through the addition of radical species or an antioxidant that decolourizes the DPPH solution. The antioxidant activity is measured by the decrease in absorption at 515 nm. In this method, 0.1 mM solution of DPPH in methanol is prepared, and 4 ml of this solution is added to 1 ml of the sample solution in methanol at 20 $\mu\text{g}/\text{ml}$, 40 $\mu\text{g}/\text{ml}$, 60 $\mu\text{g}/\text{ml}$, 80 $\mu\text{g}/\text{ml}$ and 100 $\mu\text{g}/\text{ml}$ concentrations of different solvents of aqueous and synthesized AgNPs of *P. oleracea*. Thirty minutes later, the absorbance was measured at 517 nm. A large decrease in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the extracts (Blois, 1958).

$$\% \text{ Scavenging activity} = \frac{(\text{Control OD} - \text{Sample OD})}{\text{Control OD}} \times 100$$

Cell Culture and Cytotoxicity Assays:

The MCF-7 cell lines were procured from National Centre for Cell Science (NCCS), Pune, India. These cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 2 mM glutamine, 100 U/ml penicillin, 100 $\mu\text{g}/\text{ml}$ streptomycin and 10% fetal bovine serum (FBS). Cells were cultured in 75 cm^2 cell culture flask at 37 C in a 5% CO_2 atmosphere. MCF-7 cells were cultured and seeded into 96 well plates approximately as 5×10^4 cells in each well and incubated for 24 h and 48 h. MCF-7 cells were treated with different concentrations (25 $\mu\text{g}/\text{ml}$, 50 $\mu\text{g}/\text{ml}$, 75 $\mu\text{g}/\text{ml}$, 100 $\mu\text{g}/\text{ml}$, 125 $\mu\text{g}/\text{ml}$) of aqueous and AgNPs samples. After treatment, the plates were incubated for 24 h in order to perform cytotoxic analysis. MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, a yellow tetrazole) was prepared at a concentration of 5 mg/ml and 10 μl of MTT was added in each well and incubated for 4 h. Purple color formazone crystals formed were then dissolved in 100 μl of dimethyl sulphoxide (DMSO). The crystals were

observed at 570 nm in a multi well ELISA plate reader. Optical density value was subjected to percentage of viability (Mossman, 1983).

Results

Preliminary phytochemical analysis:

The preliminary phytochemical screening of *P. oleracea* revealed the presence of carbohydrates, flavonoids, phenol, steroids and triterpenoids. Saponins, anthocyanin and fatty acids are also moderately present in aqueous whole plant extracts (Table 1).

Table 1: Preliminary phytochemical analysis of aqueous extract of *Portulaca oleracea*

S. No.	Phytocompounds	Aqueous extract
1	Carbohydrate	+++
2	Tannins	-
3	Saponins	++
4	Flavonoids	+++
5	Alkaloids	-
6	Anthocyanin	++
7	Quinones	-
8	Glycosides	-
9	Cardiac glycosides	++
10	Terpenoids	+
11	Triterpenoids	+++
12	Phenols	+++
13	Coumarins	-
14	Fatty acids	++
15	Protein	-
16	Steroids	+++

+++ strongly present; ++ moderately present; + trace amount; - not present

UV-Vis Spectral Analysis:

Figure 3 shows UV-Vis spectral record of aqueous extract and synthesized AgNPs. The sample displayed an optical absorption band peaks at about 456 nm for AgNPs, due to SPR. The increase in intensity could be due to increasing number of NPs formed as a result of reduction of Ag ions presented in the isolated compounds. Disturbances in the 200-338 nm probably indicate that capping occurred after bioreduction of AgNPs.

FT-IR Spectral Analysis:

The FT-IR spectrum identified the possible functional groups responsible for the reduction of ions and also the capping agents responsible for the stability of the biogenic NPs. In the present work, FT-IR spectra of aqueous extract and their AgNPs were analyzed and presented in Fig. 4 a and b. IR spectrum of aqueous extract and aqueous-based biosynthesized AgNPs showed characteristic peaks of C-O stretch (alcohols, carboxylic acid, esters, ethers), C=O, C=O, C Br stretch (alkyl halides) and OH stretch (H-bonded strong and broad).

Scanning Electron Microscopy (SEM):

The surface morphology of biosynthesized AgNPs was investigated by SEM analysis. The micrographs revealed more or less spherical shaped NPs with slight uniform particle size ranging between 50 and 100 nm with a few instances of large particles size and thus confirmed the formation of NPs (Fig. 5). Interestingly, close observation indicated that biosynthesized AgNPs were not in direct contact with each other even within aggregates, suggesting stabilization of biosynthesized AgNPs by the phytoconstituents.

High Resolution-Transmission Electron Microscopy (HR-TEM):

The HR-TEM images of biosynthesized AgNPs at room temperature are shown in Figure 6. The size distribution of biosynthesized AgNPs at room temperature was mostly spherical in shape with aggregation and the size ranged from 5-50 nm in size. The HR-TEM SAED pattern showed a clear Ag diffraction ring (poly dispersive), which is the indication of FCC crystalline nature of the biosynthesized AgNPs.

Cytotoxicity assay (MTT Assay):

The 24 h IC₅₀ value of MCF-7 cells was 86.459 µg/ml for AgNPs of *P. oleracea* and 127.719 µg/ml of aqueous extract of *P. oleracea* (Table 2). From the results it is obviously clear that synthesized AgNPs extract has profound effect in controlling MCF-7

Fig. 3: UV Spectral analysis of aqueous extract and AgNPs of *P. oleracea*.

Fig. 4 a: FT-IR analysis of aqueous extract of *P. oleracea*.

Fig. 4 b: FT-IR analysis of synthesized AgNPs of *P. oleracea*.

Fig. 5: Scanning Electron Microscope image of synthesized AgNPs of *P. oleracea*

Fig. 6: (A) HRTEM images and (B) SAED Pattern of synthesized AgNPs of *P. oleracea*.

Table 2: Anticancer activity of aqueous extract and their AgNPs of *P. oleracea*

Sample	Concentrations $\mu\text{g/ml}$					IC ₅₀ value $\mu\text{g/ml}$
	25	50	75	100	125	
Control	100	100	100	100	100	127.719
Aqueous extract	94.27 $\pm$ 0.347* (-5.73)	88.812 $\pm$ 1.033* (-11.188)	78.982 $\pm$ 0.390* (-21.018)	71.264 $\pm$ 0.153* (-28.736)	61.392 $\pm$ 0.577* (-38.608)	
AgNPs	85.624 $\pm$ 0.398* (-14.376)	78.136 $\pm$ 0.641* (-21.864)	68.206 $\pm$ 0.708* (-31.794)	60.246 $\pm$ 0.711* (-39.754)	42.742 $\pm$ 0.414* (-57.258)	

Values are mean + S.D. of five individual observations.

Values in parentheses are per cent change over control.

- Denotes per cent decrease over control.

*Values are significant at $P < 0.05$.

Fig. 7: Anticancer activity of aqueous extract and their AgNPs of *P. oleracea*.

Fig. 8: Anticancer activity of synthesized AgNPs of *P. oleracea*. (A) Control MCF- 7 cells; (B) 86.459 µg/ml; (C) 125 µg/ml.

Fig. 9: Anticancer activity of aqueous extract of *P. oleracea*. (A) Control MCF- 7 cells; (B) 127.719 µg/ml; (C) 125 µg/ml.

cell proliferation. Likewise, the 24 h treated aqueous extract also showed the good activity when compared to AgNPs. The data altogether depicts that the aqueous extract and their AgNPs significantly controls cell proliferation of MCF-7 cells even at lower concentration (Figs. 7-9). Hence in future, the aqueous extract and their AgNPs can be used as a potent anticancer agent.

Discussion

Cancer is a term describing conditions characterized by unscheduled and uncontrolled cellular proliferation as stated by Ponder (2001). The author also added that it is a very common disease and its incidence is increasing at an average annual rate of 1.2%. Lately, there has been improvement in the treatment strategies of cancer, which has resulted in prolonged survival of patients with chronic cancer disease.

However, there is a growing need for additional means of cancer therapy in the form of both palliative and curative treatments. The strategies available today are sophisticated and are only able to affect 50-60% of cancer patients, while the others will eventually die from the disease. Chemotherapy has been used for cancer treatment for more than 50 years; sometimes in combination with or parallel to surgery and radiotherapy. After surgical ablation of progressive cancer, metastasized tumour cells continue to progress and this is one of the faultiest effect associated with surgery. On the other hand, radioactive rays and most anticancer chemotherapeutic agents damage DNA or suppress DNA duplication to kill the rapidly growing tumour cells. At the same time, they also affect normal cells causing serious adverse effects, such as bone marrow function inhibition, bone necrosis, lung fibrosis, skin devascularization, ulceration, nausea, vomiting, renal damage and alopecia (Cassady *et al.*, 1981).

Plants represent an enormous diversity on earth, but only a small portion of that has been explored. So it is anticipated that plants can

provide potential bioactive compounds for the development of leads against various diseases indeed far greater than a wide array of selective and potent components that are needed to match the growing problems associated with cancer. Plants and natural products play an important role in medicine and provide important prototypes for the development of novel drugs (Cragg *et al.*, 1997). They serve as valuable source of compounds with wide variety of biological activities and chemical structure. Many anticancer agents have been derived from natural sources directly as pure native compounds or as semi-synthetic analogues (Moongkarndi *et al.*, 2004).

Conclusion

In conclusion, our results showed that the synthesized silver nanoparticles possess potent larvicidal activity against MCF-7 cell lines and significantly reduced the cell viability, and altered the cellular morphology of MCF-7 cells in a concentration dependent manner. The data revealed that the exposure to methanol extract was more effective in MCF-7 cells. Further molecular studies are needed to elucidate the mechanisms of action of the methanol extract on human breast cancer cells. Hence the plant extracts as well as its nanoparticles can be used as a potent anti-cancer agent.

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