IJPSR (2021), Volume 12, Issue 9 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 15 September 2020; received in revised form, 08 February 2021; accepted, 22 June 2021; published 01 September 2021

SYNTHESIS OF SILVER NANOPARTICLES USING LEAVES AND STEM BARK OF *PTEROCARPUS INDICUS* AND ITS ACTIVITY ON HUMAN BREAST CANCER CELL LINE (MCF-7)

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Keywords:

Leaves and stem bark of *Pterocarpus indicus*, Silver nanoparticles, MTT assay, MCF-7 cell line, Ethanol extract

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ABSTRACT: In the present study, synthesis of silver nanoparticles (AgNPs) using leaves and stem bark of *Pterocarpus indicus* were tested against human breast cancer cell line (MCF-7) separately. The synthesized AgNPs were characterized using UV-visible spectroscopy, X-ray powder diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), Scanning Electron Microscope (SEM), and zeta potential. The synthesized silver nanoparticles were checked with the color variation, and it was confirmed by UV-vis spectral analysis. The morphology of the synthesized nanoparticles was analyzed using SEM. The XRD was done to find out the crystalline structure of the compound. FTIR measurements are carried out to identify the possible biomolecules responsible for capping and efficient stabilization of the silver NPs synthesized using *Pterocarpus indicus*. The synthesized leaves-AgNPs and stem bark-AgNPs of *Pterocarpus indicus* were tested against the MCF-7 cell line to determine cell viability. Results showed that the stem bark-AgNPs shows more stability than leaves-AgNPs due to their higher negative zeta potential (-16.3 mV) than nanoparticles biosynthesized from leaves-AgNPs (−13.1 mV). The synthesized silver nanoparticles inhibited the proliferation of human breast cancer cell lines with an IC_{50} value of (126.4 µg/ml) for stem bark-AgNPs and for leaves-AgNPs (54.60 µg/ml). This study concluded that synthesized AgNPs using *Pterocarpus indicus* have potential anticancer activity.

INTRODUCTION: Nanotechnology is the appearing one in the field of science. In biological field, nanotechnology provides rapid research in the field of genetics. Silver nanoparticles are majorly used for its distinctive properties in catalysis, biosensing, chemical sensing, photonics and pharmaceuticals [1]. In physical and chemical methods, the synthesis of nanoparticles requires temperature, pressure and also energy is consumed.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(9).4807-16

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(9).4807-16

In the Biological method, the synthesis of silver nanoparticles using the plant is more advantageous because of its low energy consumption and environment friendly [2]. Silver nanoparticles used in wide range of application such as antimicrobial, anti-inflammatory [3], antiviral [4] and anti-diabetic [5] and also involved in the prevention of diabetic wound healing (ointments) [6].

Cancer is a proliferating disease combining physical, environmental, metabolic, chemical and genetic factors [7]. In US country, Breast cancer is the second leading cause of death in women. An estimated 39,620 breast cancer death and 232,340 new cases are expected among women in 2013 [8]. In 2019, breast cancer has been diagnosed among 268,600 people in US women, and 41,760 people

were died. The mortality rate for breast cancer is projected to decrease from 16.1 in 2015 to 14.7 in 2030. Breast cancer cell targeting drug development without affecting normal cells is a challenging task in the field of drug discovery for cancer [9]. Cytotoxic agents are expensive and induce severe side effects [10, 11]. Currently, a variety of cytotoxic agents have been used in the treatment of breast cancer, such as doxorubicin, cisplatin, and bleomycin. Although usage of drugs provides beneficial effects, but the efficacy and demerits are uncertain [12]. In the olden centuries, medicinal plants are used as effective anticancer agents [13]. Several medicinal plant species and their phytochemicals inhibit the progression and development of cancer [14].

Pterocarpus indicus is belonging to the family of Fabaceae, and its Tamil name is vengai maram [15]. It is an ayurvedic herb plant used in the treatment of many ailments such as CNS activity, antidiabetic, hepatoprotective, anti-inflammatory and anti-oxidant, cardiotonic, anti analgesic and antioxidant, antimicrobial, anti-hyperglycemic and hypoglycemic, cytotoxic, ant proliferative and anti-inflammatory, anti-hyperinsulinaemic and antihypertriglyceri-demia, anti-cataract, antifungal activity [16]. Their phytochemical constituents like tannin, phloba-tannin, saponin, flavonoids, steroids, terpenoids, cardiac glycosides, leucoanthocyanin, anthocyanin, anthroquione, coumarin, glycosides, phenol, xanthoprotein, alkaloids, emodine, carbohydrate. *Pterocarpus indicus* is a medicinally valuable species widely distributed in the region pacific southwest and northern and Asia east and southeast to native [17, 18]. So, it is necessary to find novel therapeutic agents against cancer, which are biocompatible and cost-effective. Therefore, this study was designed to synthesize AgNPs using leaves and stem bark of *Pterocarpus indicus* and to evaluate potential toxicity and the general mechanism of synthesized AgNPs in human breast cancer cells (MCF-7 cells) separately.

MATERIALS AND METHODS:

Collection of Plant Material: The *Pterocarpus indicus* bark and leaves were collected in the month of December from the Senthankudi Village, Pudukkottai, Tamil Nadu, India.

The plant was identified and authenticated by Dr. S. John Britto, Director, Rapinat herbarium, St. Joseph College, Tiruchirappalli, Tamil Nadu, for identifying the plants.

Chemical Reagents and Instruments Required:

Chemicals: Silver nitrate, DMEM medium, Fetal Bovine Serum (FBS) and antibiotic solution were from Gibco (USA), DMSO (Dimethyl sulfoxide) and MTT (3-4,5 dimethylthiazol-2yl-2,5-diphenyl tetrazolium bromide) (5 mg/ml) were from Sigma, (USA), 1X PBS was from Himedia, (India). 96 well tissue culture plates and wash beakers were from Tarson (India).

Instruments: Lambda 35, Perkin Elmer Spectro-photometer, Malvern zetasizer version 2.2., XPERT-PRO Machine and TEM, JEOL-JEM 2100

Ethanolic Extract of Plant Preparation: The leaves and stem bark of *Pterocarpus indicus* plant were collected and washed with fresh water. After, it was dried in a shaded region for 35-40 days and made into fine powder. In a dry beaker, the absolute amount of powder and few amounts of ethanol were added. These solutions were shifted into another beaker, stirred with a glass rod, and closed with a watch glass. Boiling this solution for 20-30 min; the color changes occur from slight green into dark green in color and cooled at room temperature for 1 h. The extract was filtered using Whatman no. 41 filter paper in a clean beaker. Finally, these stock solutions were transferred into a brown bottle and then stored in a cooled place for further study [19].

Optimization and Synthesis of Silver Nanoparticles: Ethanolic extract of each leaves and stem bark of *Pterocarpus indicus* were used as a stock solution. A perfect amount of silver nitrate was weighed in a 50ml standard flask and liquefied with deionized water. Aluminum foil was used to close the flask for the preclusion of photochemical reactions. Different concentration of the stock solution (25µl, 50µl, 75µl, and 100µl) was mixed with 1mM of silver nitrate solution without any contamination. Then, this solution was kept at room temperature for the formation of silver nanoparticles. A color change occurs from colorless to red-brown, which indicates the formation of silver nanoparticles transparently without any agglomeration and is highly stable in nature [20].

Characterization Techniques:

UV-visible analysis: UV-Visible spectroscopy (UV-Vis) technique is used to measures the optical properties (size, shape, concentration, agglomeration state, and refractive index) of the nanoparticles by the eradication (scatter + absorption) of light passing through a sample. Hence, nanoparticles were identified, characterized and studied by UV- Visible spectroscopy [21, 22].

FTIR Analysis: Fourier Transform Infrared Spectroscopy is otherwise called FTIR Analysis or FTIR Spectroscopy. The test sample can be scanned by infrared light, and chemical properties like organic, polymeric, and inorganic materials were observed by this method. Fourier Transform Spectrometer absorbs infrared spectra within the range of 400-4500 cm^{-1}. At a particular frequency, multiple functional groups may be absorbed, and it gives rise to different characteristic absorptions.

XRD: X-ray diffraction (XRD) analysis is used to study the nanomaterials (with structural features in the range of 1-100 nm). The structure of nanomaterials has been probed by XRD method. The position of values of the product (crystallinity or amorphous nature) can be identified by this technique. With respect to d-spacing values; the fingerprint regions of relative intensity are found in XRD analysis.

SEM Analysis: Samples were mounted on 12 mm aluminium specimen stubs with double-sided carbon tape, coated with gold-palladium, and examined with an FEI Quanta 250 FEG SEM operating at 10 kV.

Zeta Potentiometer: Size and zeta potential of the silver nanoparticles were determined by Malvern Zetasizer ZEN 3600 (United Kingdom). This instrument allows the measurement of particle-sized distribution in the range 2 nm–3 nm.

Cell Culture: MCF-7 (Human breast cancer cells) cell lines were cultured in liquid medium (DMEM) supplemented 10% Fetal Bovine Serum (FBS), 100 ug/ml penicillin, and 100 µg/ml streptomycin, and maintained under an atmosphere of 5% CO_2 at 37 °C.

MTT Assay: The leaves and stem bark of *Pterocarpus indicus* were tested for *in-vitro* cytotoxicity, using MCF-7 cells by 3-(4,5-dimethylthiazol-2-yl)- 2, 5- diphenyltetrazolium bromide (MTT) assay separately. Briefly, the cultured MCF-7 cells were harvested by trypsinization, pooled in a 15 ml tube. Then, the cells were plated at a density of 1×10^5 cells/ml cells/well (200 µL) into a 96-well tissue culture plate in a DMEM medium containing 10 % FBS and 1% antibiotic solution 24-48 hour at 37 °C. The wells were washed with sterile PBS and treated with various concentrations of the sample in a serum-free DMEM medium. Each sample was replicated three times, and the cells were incubated at 37 °C in a humidified 5% CO2 incubator for 24 h. After the incubation period, MTT (20 µL of 5 mg/ml) was added into each well after the incubation period. The cells were incubated for another 2-4 h until purple precipitates were clearly visible under an inverted microscope. Finally, the medium and MTT (220 µL) were aspirated off the wells and washed with 1X PBS (200 µl). Furthermore, to dissolve formazan crystals, DMSO (100 µL) was added, and the plate was shaken for 5 min. The absorbance for each well was measured at 570 nm using a microplate reader (Thermo Fisher Scientific, USA). The percentage cell viability and IC50 value were calculated using Graph Pad Prism 6.0 software (USA) 23 [24].

RESULTS:

Visual Color Change and UV-Vis Spectroscopy: In this experiment, the addition of ethanol extract of leaves and stem bark of *Pterocarpus indicus* separately into the glass vial containing $AgNO_3$ led to the change in color from colorless to reddish-brown indicates the presence of silver nanoparticles. UV spectra observed plasma resonance band at the range of 435 nm for the leaves-AgNPs and at the range of 439 nm for the stem bark-AgNPs **Fig. 1**.

Functional Group Determination using FT-IR Spectroscopy: The FT-IR analysis of synthesized Ag NPs is presented in **Fig. 2**. The following peaks are observed in the leaves-AgNPs; the medium band primary amine at 3438.11 cm^{-1} corresponds to N-H stretching vibrations. The strong band Isothiocyanate at 2085.17 cm^{-1} corresponds to N=C=S stretching vibrations. The medium band Alkene at 1637.86 cm^{-1} corresponds to C=C stretching vibrations, Amine at 1109.08 cm^{-1}

corresponds to C-N stretching vibrations. The Strong band Halo compound at 670.15 cm^{-1} corresponds to C-Br stretching vibrations **Fig. 2a**.

For the stem bark-AgNPs, the strong band primary amine at 3441.16 cm^{-1} corresponds to 3441.16 cm^{-1} corresponds to broad O-H stretching alcohol. The Strong band Isothiocyanate at 2077.79 cm^{-1} corresponds to N=C=S stretching vibrations. The Medium band Alkene at 1634.81 cm^{-1} corresponds to C=C stretching conjugated alkene, and amine at 1405.54 cm^{-1} corresponds to O-H bending alcohol. The strong band Alcohol at 1114.45 cm^{-1} corresponds to C=O secondary alcohol, Anhydride at 1030.7 cm^{-1} corresponds to CO-O-CO anhydride, and Halo compound at 662.74 cm^{-1} corresponds to C-Br stretching vibrations **Fig. 2b**. Analysis of these spectra strongly suggested the presence of flavonoids and phenols, which were mainly responsible for the formation of silver nanoparticles by reducing silver nitrate.

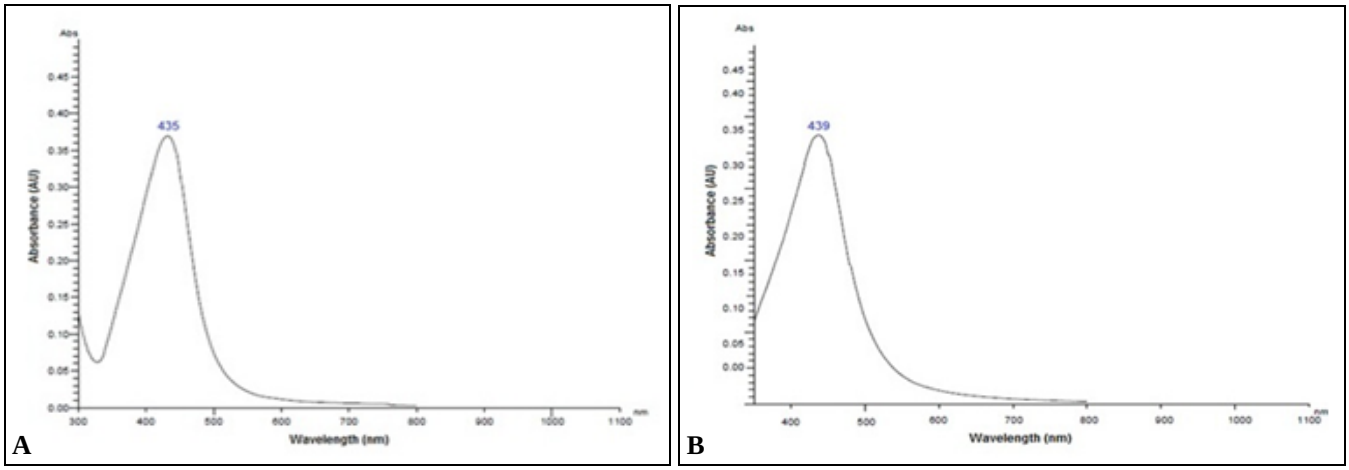

FIG. 1: UV-VIS SPECTRUM OF SYNTHESIZED SILVER NANOPARTICLES USING *PTEROCARPUS INDICUS* (A) LEAVES-AgNPs (B) STEM BARK-AgNPs

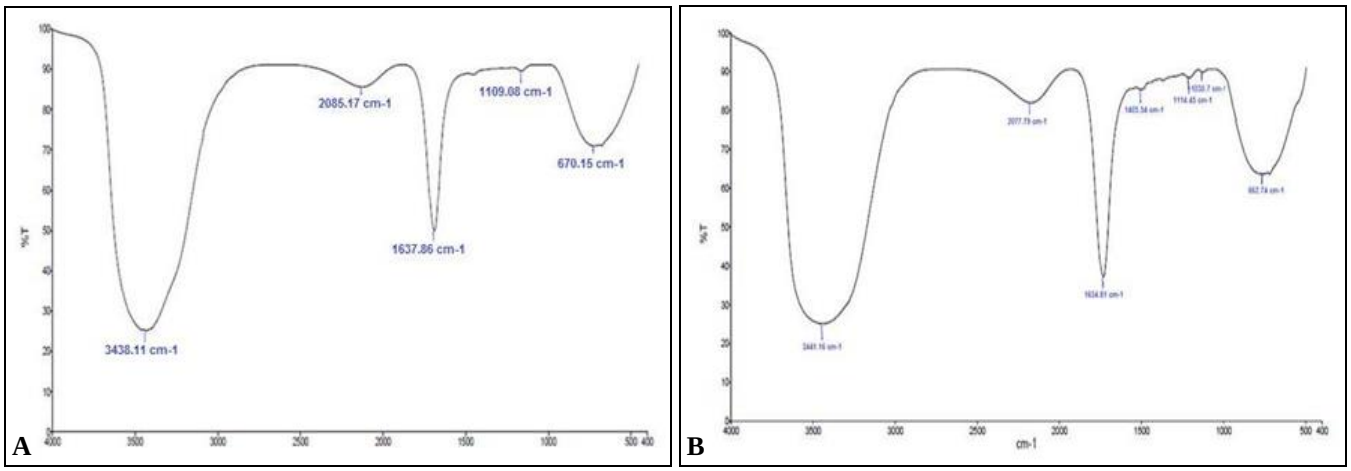

FIG. 2: UV-VIS SPECTRUM OF SYNTHESIZED SILVER NANOPARTICLES USING *PTEROCARPUS INDICUS* (A) LEAVES-AgNPs (B) STEM BARK-AgNPs

X-Ray Diffraction (XRD): The XRD spectrum was recorded to confirm the crystalline structure of synthesized AgNPs using the leaves and stem bark of *Pterocarpus indicus*. The result is shown in **Fig. 3**. The diffraction peaks were obtained by leaves-AgNPs is observed at 38.4024, 46.3896, 65.1721 and 78.568 in the 2Ɵ range **Fig. 3a**. The obtained XRD pattern for silver nanoparticles synthesized using *Pterocarpus indicus* bark extract showed the characteristic peaks 38.7965, 44.7206, 65.0686, and 78.2134 in the 2Ɵ range **Fig. 3b**.

The peaks can be indexed to the (111), (200), (220) and (311) reflection of the face-centered cubic structure of metallic silver, which suitably matched the standard diffraction data with those reported for silver by joint committee on powder diffraction standards (JCPDS) FILE NO: 040783.

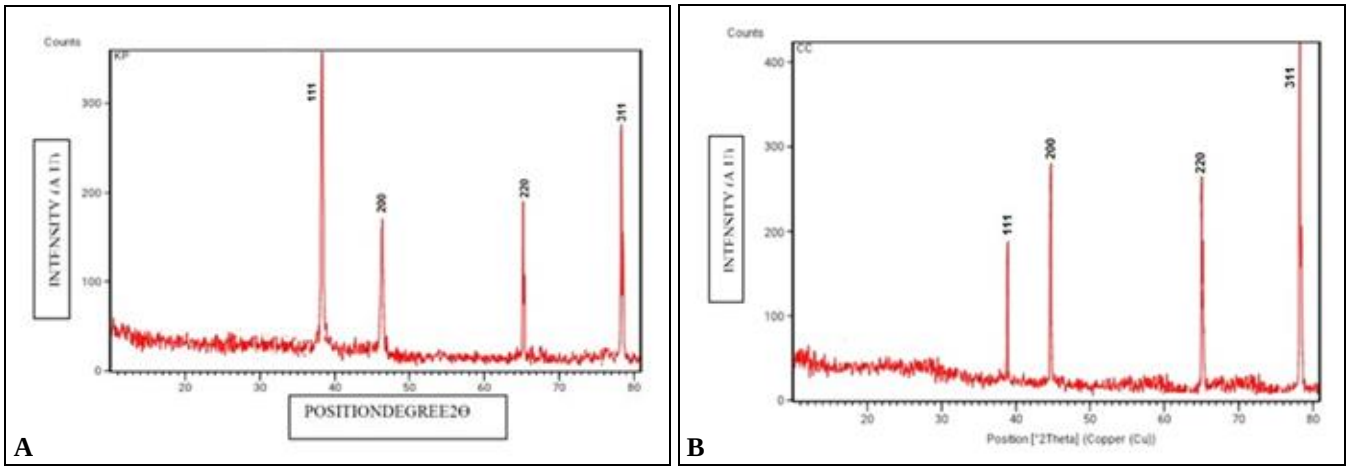

FIG. 3: XRD ANALYSIS OF SYNTHESIZED SILVER NANOPARTICLES USING *PTEROCARPUS INDICUS* (A) LEAVES-AgNPs (B) STEM BARK-AgNPs

SEM Image: The SEM image is employed to predict the size and morphology of resultant silver nanoparticles using leaves and stem bark of *Pterocarpus indicus*. The size (diameter) of the nanoparticles lies between 37.9-124.5 nm region in case of leaves-AgNPs **Fig. 4a** and 98.70-126 nm in case of stem bark-AgNPs sample **Fig. 4b**, the average size of the nanoparticles is ~ 200 nm, whereas the shapes were spherical and cubic.

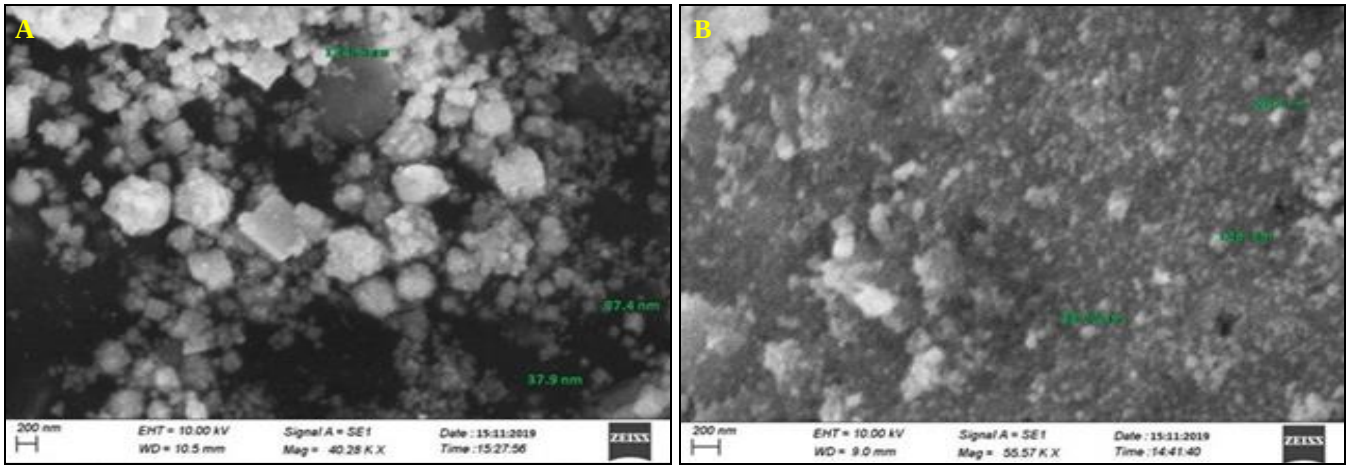

FIG. 4: SEM PHOTOGRAPH OF SYNTHESIZED SILVER NANOPARTICLES USING *PTEROCARPUS INDICUS* (A) LEAVES-AgNPs (B) STEM BARK-AgNPs

Particle Size Distribution and Zeta Potential Studies: Size distribution analysis was performed using dynamic light scattering to know the size of the biosynthesized silver nanoparticles. Dynamic light scattering results affirmed an average diameter of the biosynthesized AgNPs was in the nanometer range. The intensity weighted particle size distribution histograms are obtained from the leaves-AgNPs exhibited polydisperse mixture with the size ranging of 1842 nm with polydispersity index of 0.247 **Fig. 5a,** and for the stem bark-AgNPs exhibited polydisperse mixture with the size ranging of 385 nm with polydispersity index of 0.063 **Fig. 6a**. Thus, the particle size distribution studies confirm that the Ag particle has formed in nanosize and well match with the average particle diameter range obtained from SEM studies.The zeta potential is used to depict the surface charge and stability of synthesized silver nanoparticles using *Pterocarpus indicus*. As shown in **Fig. 6b**, the biosynthesized leaves-AgNPs had a negative charge with a zeta potential value −13.1 mV.

The biosynthesized stem bark-AgNPs had a negative charge with a zeta potential value −16.3 mV **Fig. 6b**. This zeta potential value falls within the range of −20 to −30 mV is considered as moderately stable, which clearly indicated that the synthesized S-AgNPs were moderately stable in nature.

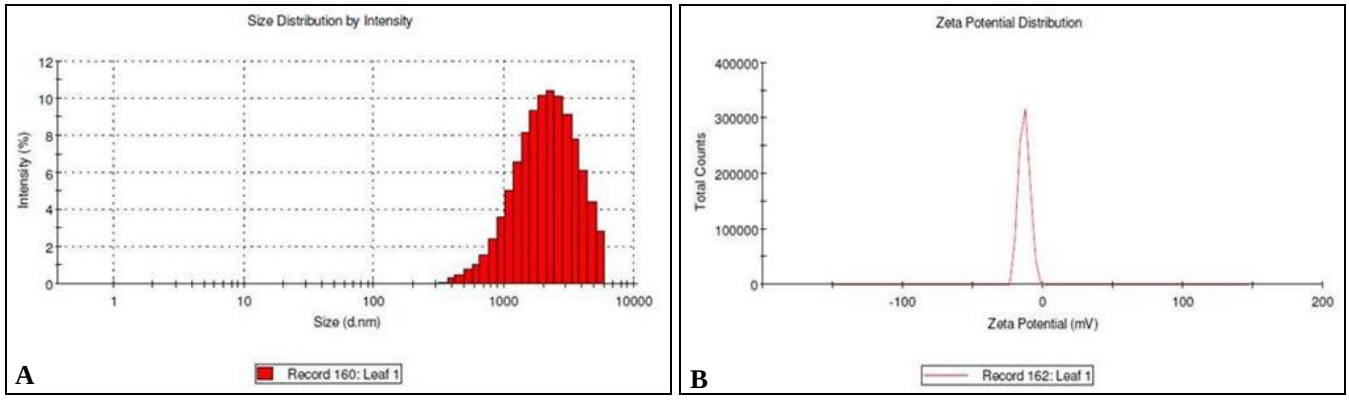

FIG. 5: (A) PARTICLE SIZE DISTRIBUTION, AND (B) ZETA POTENTIAL MEASUREMENT OF THE BIOSYNTHESIZED AGNPS USING *PTEROCARPUS INDICUS* LEAVES EXTRACT

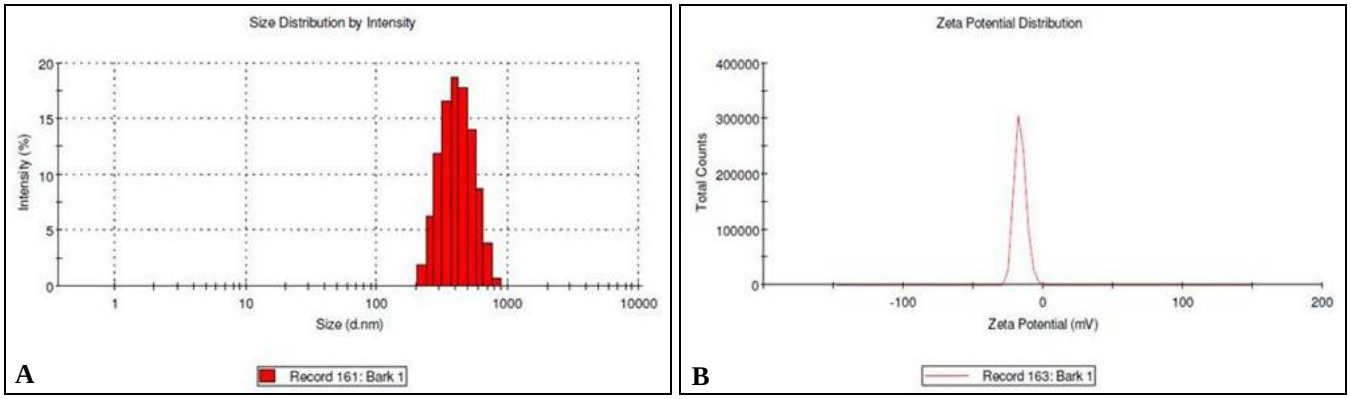

FIG. 6: (A) PARTICLE SIZE DISTRIBUTION, AND (B) ZETA POTENTIAL MEASUREMENT OF THE BIOSYNTHESIZED AgNPs USING *PTEROCARPUS INDICUS* STEM BARK EXTRACT

Cytotoxicity Activity: The Cytotoxicity study was carried out for the synthesized silver nanoparticles using leaves and stem bark of *Pterocarpus indicus* separately. These leaves and stem bark AgNPS were screened for its cytotoxicity against MCF -7 breast cancer cell lines at different concentrations 10-500 µg/ml to determine the IC_{50} (50% growth inhibition) by MTT assay was summarized in **Table 2**. The table's shows that the stem bark-AgNPs was found more cytotoxic as compared to the leaves-AgNPs on MCF-7 cancer cell lines tested as indicated by the IC_{50} values. Cytotoxicity was observed against MCF-7 cell line IC_{50} value is 54.60 (µg/ml) for the leaves-AgNPs and for the stem bark-AgNPs is 126.4 (µg/ml)respectively).

From the results of MTT analysis, it is concluded that stem bark-AgNPs of *Pterocarpus indicus* showed a maximal antiproliferative effect on breast cancer cell lines (MCF-7) when compared to the leaves-AgNPs.

From this cell viability assay, the synthesized silver nanoparticles leaves have more capacity to kill MCF-7 cells when compared to stem bark of *Pterocarpus indicus*. There is no drastic change in leaves-AgNPs treated cells when the time is increased, whereas the synthesized stem bark-AgNPs treated cells cause variation in cell viability when the time is increased at 500 and 400 µg/ml **Graph 1.**

TABLE 1: OPTICAL DENSITY AND CELL VIABILITY OF SYNTHESIZED SILVER NANOPARTICLES USING LEAVES AND STEM BARK OF *PTEROCARPUS INDICUS* ON MCF 7 CELL LINE BY MTT ASSAY

S. no.	Tested sample concentration (µg/ml)	Leaves-AgNPs			Stem bark-AgNPs		
		Absorbance (Optical density)	Cell Viability (%)	(IC_{50})	Absorbance (Optical density)	Cell Viability (%)	(IC_{50})
1	Control	0.464	100		0.422	100	
2	10 µg/ml	0.456	98.2	54.60	0.348	82.61	
3	20 µg/ml	0.451	97.19	(µg/ml)	0.329	78.03	
4	40 µg/ml	0.434	95.04		0.320	75.98	
5	60 µg/ml	0.407	92.24		0.302	71.72	126.4
6	80 µg/ml	0.369	79.59		0.253	59.94	(µg/ml)

7	100 µg/ml	0.318	68.6	0.235	55.76
8	200 µg/ml	0.295	63.57	0.196	46.52
9	300 µg/ml	0.276	59.55	0.165	39.09
10	400 µg/ml	0.219	47.19	0.11	26.06
11	500 µg/ml	0.136	29.37	0.095	22.58

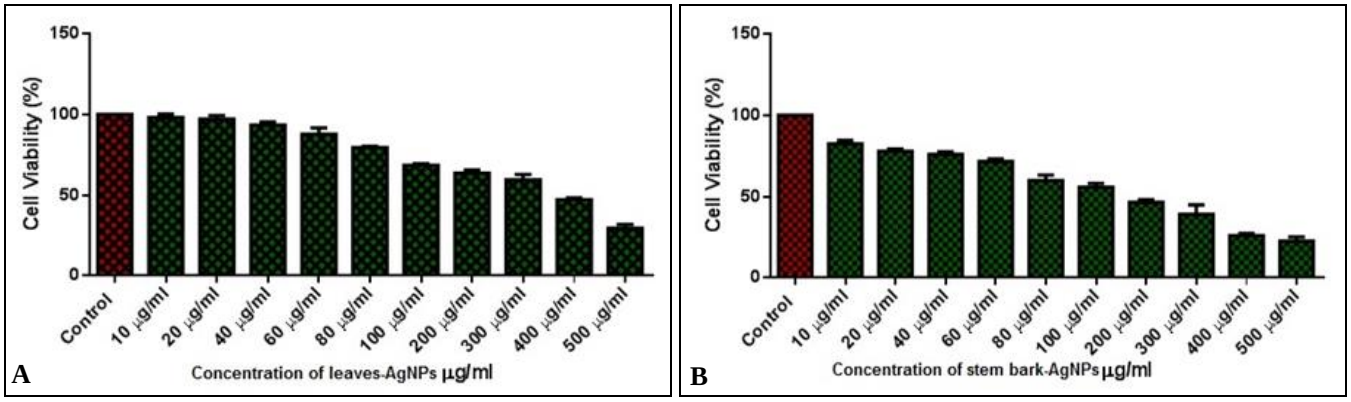

FIG. 7: PERCENTAGE OF CELL GROWTH INHIBITION OF SYNTHESIZED SILVER NANOPARTICLES USING LEAVES (A) AND STEM BARK (B) OF *PTEROCARPUS INDICUS* ON MCF 7CELL LINE BY MTT ASSAY

The morphological changes of the cell lines treated cells with various concentrations of leaves-AgNPs and stem bark-AgNPs of *Pterocarpus indicus* was incubated for 24 h and compared with the untreated cells. Compared to control cells after the incubation period, the morphology of the leaves-AgNPs and stem bark-AgNPs treated cancer cells significantly changed and appeared less uniform with the loss of membrane integrity, although still intact at lower concentrations. Whereas at higher concentrations, the leaves-AgNPs and stem bark-AgNPs treated cells showed remarkable differences with the control group. The significant changes such as loss of intact membrane, karyopyknosis, cell detachment from the plate, and change of morphological features were evident when compared to untreated cells. The most identifiable morphological features of apoptosis were observed by inverted light microscopy in the leaves-AgNPs, and stem bark-AgNPs treated cells **Fig. 8**. The treated cells appeared like cells undergoing apoptosis with prominent features such as detaching from the culture plate, cytoplasmic condensation, cell shrinkage and condensation and aggregation of the nuclear chromatin, and loss of contact with neighbouring cells **Fig. 9**. However, the untreated cells appeared normal and were confluent.

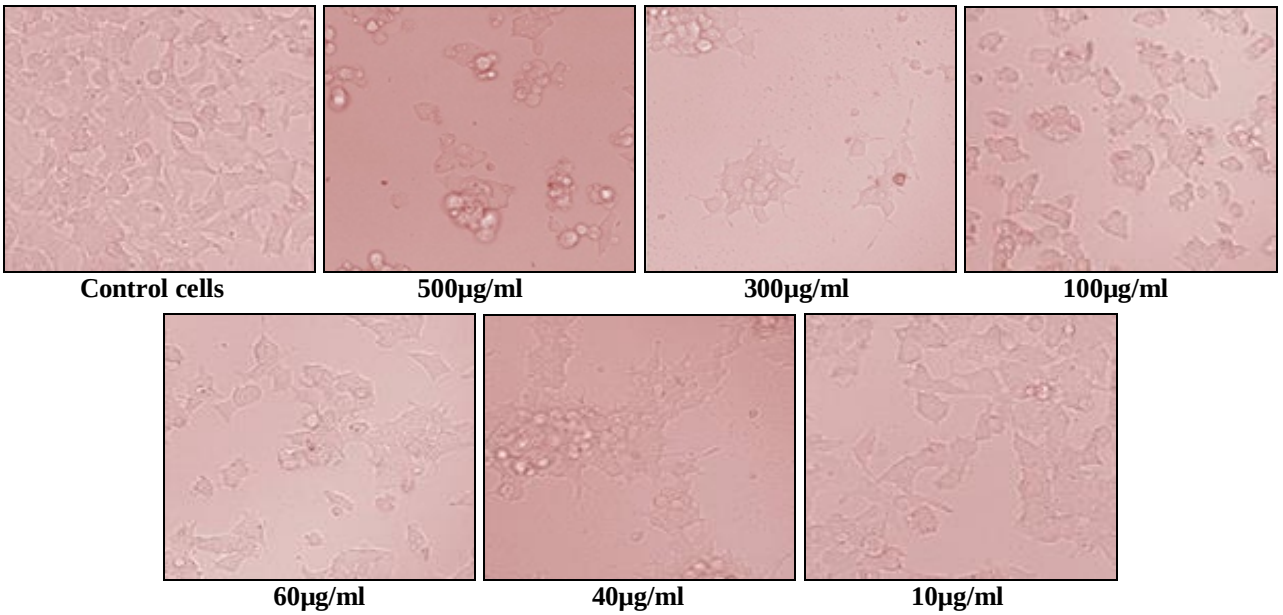

FIG. 8: CYTOTOXIC EFFECT OF SYNTHESIZED SILVER NANOPARTICLES USING LEAVES OF *PTEROCARPUS INDICUS* ON MCF 7 CELL LINE BY MTT ASSAY

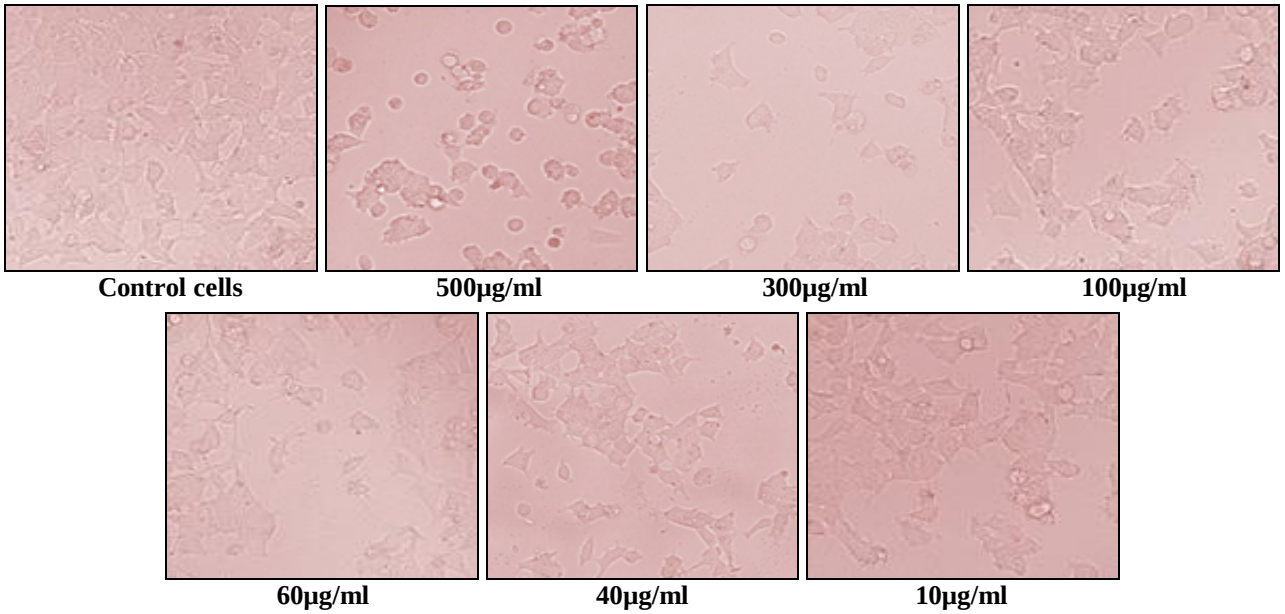

FIG. 9: CYTOTOXIC EFFECT OF SYNTHESIZED SILVER NANOPARTICLES USING STEM BARK OF *PTEROCARPUS INDICUS* ON MCF 7 CELL LINE BY MTT ASSAY

DISCUSSION: In the current study, the human breast cancer cell line (MCF-7) activity of *Pterocarpus indicus* and cell viability variations of synthesized leaves-AgNPs and stem bark-AgNPs were noted. The silver nanoparticle was synthesized using leaves and stem bark of *Pterocarpus indicus*. The color was changed from light green to dark brown for leaves and from yellow to brown color for the bark of *Pterocarpus indicus.* This clearly demonstrated that the synthesis of silver nanoparticles formed. The synthesis was confirmed by a UV-vis spectrophotometer. It has been reported that the silver nanoparticle starting from light yellow to dark brown. The absorption spectra of silver nanoparticle twisted in the reaction media have absorbance peak at 420 nm [25]. The development of silver nanoparticles using *Pterocarpus indicus* was viewed by the color change [26]. It has been reported that the XRD patterns of dried AgNPs synthesized using fluoresced peaks at 2Φ values of 21.64º, 29.48º, 38.84º, 43.28º and 53.48º assigned to the (200), (101), (144), (202) and (311) planes of a faced center cubic lattice of silver [27]. Therefore, XRD results suggested that crystallization of the bioorganic phase occurs on the plane of the AgNPs [28].

The FTIR analysis was carried out to find the presence of biomolecules present in the synthesized silver nanoparticles. Higher peaks at 3438 and 3441 cm-1 might be attributed to bounded hydroxyl (OH) of alcohol /phenols or amine (-NH) groups of leaves-AgNPs and stem bark-AgNPs, respectively. Previous studies suggested that these bonds could be due to the hydroxyl group stretching in proteins, enzymes or polysaccharides found in their extracts of *Pterocarpus indicus* [29]. Lower bands at 670 and 662 cm-1 are suggested to be due to alkylhalides such as C-Cl stretching found in anthocyanin flavonoids that are present in those leaves' extracts [30]. Therefore, free groups present in the proteins and flavonoids, such as anthocyanins and gallic acid, found in ethanolic extracts of *Pterocarpus indicus* were responsible for silver nitrate reduction to silver nanoparticle and capping nanoparticles for stabilization and prevent their aggregation in the medium have been previously reported [31, 32, 33]. SEM was to find the morphology of the synthesized silver nanoparticles reported earlier that the SEM images for the biosynthesized silver nanoparticles cluster in shape with an average size ranging from 20-100 nm. This image confirmed the formation of nanoparticles capped with its biomoites due to surface Plasmon resonance [34]. The previous studies reported that a high negative potential value increases negative-negative repulsion between silver nanoparticles that in turn assists their long-term stability, gold colloidal nature and high dispersity [35]. Stem bark-AgNPs appear larger in size than those reported in previous studies that might be due to its low negativity, leading to higher attraction force and, in turn, lead to nanoparticle aggregation [36].

The synthesized leaves-AgNPs and stem bark-AgNPs of *Pterocarpus indicus* were tested for cytotoxicity, cell viability, and morphological observation. The present results agreed with previous studies showing the in vitro cytotoxic effect of biosynthesized AgNPs using different plant extracts such as *Annona squamosa* against the breast cancer MCF-7 cell line [37]. In addition, many herbs contain a variety of phenolics, phytosterols, triterpenes, flavonoids, saponins, and carotenoids, which have been shown from studies of legumes, fruit, and vegetables to be cancer chemoprotective [38]. It was also reported that phenolics reduced the amount of cellular protein and mitotic index and the colony formation during cell proliferation of cancer cells. The presence of a 4-carbonyl group of the flavonoid molecule also contributes to anticancer activity.

In addition, the presence of a 2,3-double bond in flavonoid molecules correlates with mitochondrial damage and cancer cell death [39]. A previous study demonstrated that AgNPs from *S. striata* flower extract may be a potential therapeutic agent for human breast cancer treatment [40]. Silver nanoparticles can be used as anticancer agents with minimal effect on normal tissues [41]. The present studies suggest that the AgNPs are synthesized as well as stabilized by *Pterocarpus indicus*. These synthesized nanoparticles highly inhibit the growth of cancer cells (MCF-7) have great importance as a therapeutic agent in preventing or lowering oxidative stress related to degenerative diseases, such as breast cancer.

CONCLUSION: In the present study, a simple and economical approach has been attempted to obtain a green eco-friendly synthesis of silver nanoparticles which was obtained from bio-reduction of *Pterocarpus indicus* leaves and stem bark extracts with $AgNO_3$ solution. Silver nanoparticles synthesized by the green chemistry approach reported in the present study may have potent applications in human breast cancer. Synthesized AgNPs from the plant extracts is characterized specifically using UV-Visible spectroscopy, zeta potential for particle size analysis, and SEM, whereas protocol to produce uniform-sized nanoparticles has to be standardized for specific applications. The synthesized silver nanoparticles are spherical, with sizes in the ranges from 37.9-126 nm. Additionally, the synthesized silver nanoparticles showed significant enzyme *in-vitro* cytotoxicity activity against breast cancer cell line (MCF-7), so the leaves AgNPs and the stem bark-AgNPs of *Pterocarpus indicus*, which are responsible for anticancer activity, have to be done for the usage of anticancer agent.

ACKNOWLEDGEMENT: Nil

CONFLICTS OF INTEREST: The authors declare that they have no conflict of interest. It has not been published elsewhere. That it has not been simultaneously submitted for publication elsewhere, all authors agree to the submission to the Journal.

REFERENCES:

1. Sarkar R, Kumabhakar P and Mitra AK: Green synthesis of silver nanoparticles and its optical properties. Digest J. Nanometer. Biostruct 2010; 5: 491-96.
2. Pandian N and Chidambaram S: Antimicrobial, cytotoxicty and anticancer activity of silver nanoparticles from *Glycyrrhiza glabra*. Int J Pharm Sci Res 2015; 41: 1633.
3. David L, Moldovan B and Vulcu A: Green synthesis, characterization and antiinflammatory activity of silver nanoparticles using European black elderberry fruits extract. Colloids Surf B 2014; 122: 767-77.
4. Zodrow K, Brunet L and Mahendra S: Polysulfone ultrafiltration membranes impregnated with silver nanoparticles show improved biofouling resistance and virus removal. Water Res 2009; 43(3): 715-23.
5. Kiran MV and Murugesan S: Biogenic silver nanoparticles by Halymenia poryphyroides and its *in-vitro* anti-diabetic efficacy J of Chem and Pharma Res 2013; 5(12): 1001-08.
6. Manish M, Hemant K and Kamlakar T: Diabetic delayed wound healing and the role of silver nanoparticles. Digest J of Nanomaterials and Biostructures 2008; 3(2): 49-54.
7. Shahedur R, Faizus S and Asif I: *In-vitro* antioxidant and anticancer activity of young *Zingiber officinale* against human breast carcinoma cell lines. BMC Complementary and Alternative Medicine 2011; 11: 76.
8. Lupu R, Cardillo M and Cho C: The significance of heregulin in breast cancer tumor progression and drug resistance. Breast Cancer Res and Trea 1996; 38: 57-66.
9. Schwartsmann G, Ratain MJ, Cragg GM, Wong JE, Saijo N and Parkinson DR: Anticancer drug discovery and development throughout the world. Journal of Clinical Oncology 2002; 20: 47S-59S.
10. Kim DW, Hong GH, Lee HH, Choi SH, Chun BG, Won CK, Hwang IK and Won MH: Effect of colloidal silver against the cytotoxicity of hydrogen peroxide and naphthazarin on primary cultured cortical astrocytes. Neuroscience 2007; 117: 387-400.
11. Yeruva L, Elegbede JA and Carper SW: Methyl jasmonate decrease membrane fluidity and induces apoptosis via tumour necrosis factor receptor 1 in breast cancer cells. Anti-cancer Drug. 2008; 19: 766-76.
12. Ahmad R, Ahmad N, Naqvi AA, Shehzad A, Al-Ghamdi MS. Role of traditional Islamic and Arabic plants in cancer therapy. J Tradit Complement Med 2017; 7(2): 195-204.

13. Franco-Molina MA, Mendoza-Gamboa E and Sierra-Rivera CA: Antitumor activity of colloidal silver on MCF-7 human breast cancer cells. Journal of Experimental and Clinical Cancer Research 2010; 29(148): 1-7.
14. Kim JS, Kuk E and Yu KN: Antimicrobial effects of silver nanoparticles. Nanomedicine 2007; 3: 95-101.
15. Kim MR, Choi HK, Cho KB, Kim HS and Kang KW: Involvement of Pin1 induction in epithelial-mesenchymal transition of tamoxifen-resistant breast cancer cells. Cancer Science 2009; 100(10): 1834-41.
16. Wang S, Ding S, Zhang X and Zhang L: A new medicinal plant Pterocapus indicus]. Zhongyaocai= Zhongyaocai= J of Chinese Medicinal Materials 1997; 20(7): 330-32.
17. Ragasa CY, De Luna RD and Hofilena JG: Antimicrobial terpenoids from *Pterocarpus indicus*. Natural Product Research 2005; 19(4): 305-09.
18. Hofilen´a JG and Ragasa CY: A monoteeroene from *Pterocarpus indicus*. Kimika 2002; 18: 58.
19. Deepti R, Sushila R, Permender R, Aakash D, Sheetal A and Dharmender R: HPTLC densitometric quantification of stigmasterol and lupeol from *Ficus religiosa*. Arab J Chem 2015; 8: 366-71.
20. Ahmed S, Saifullah, Ahmad M, Swami BL and Ikram S: Green synthesis of silver nanoparticles using *A. indica* aqueous leaf extract. J of Rad Res & App Sci 2016; 9: 1-7.
21. Subbaiya R, Lavanya RS, Selvapriya K and Selvam MM: Green synthesis of silver nanoparticles from *Phyllanthus amarus* and their antibacterial and antioxidant properties. Int J Curr Microbiol App Sci 2014: 3: 600-06.
22. Krishnaraj C, Jagan EG, Rajasekar S, Selvakumar P and Kalaichelvan PT: Synthesis of silver nanoparticles using *Acalypha indica* leaf extracts and its antibacterial activity against water borne pathogens. Colloids Surf B Biointerfaces 2010; 76: 50-56.
23. Manikandan R, Beulaja M, Arulvasu C, Sellamuthu S, Dinesh D and Prabhu D: Synergistic anticancer activity of curcumin and catechin: an *in-vitro* study using human cancer cell lines. Microsc Res Tech 2012; 75: 112-6.
24. Babu G, Arulvasu C, Prabhu D, Jegadeesh R and Manikandan R: Biosynthesis and characterization of silver nanoparticles from *Datura inoxia* and its apoptotic effect on human breast cancer cell line MCF-7. Materials Letters 2014; 98-102.
25. Ahmed S, Saifullah, Ahmad, M, Swami BL and Ikram S: Green synthesis of silver nanoparticles using *Azadirachta indica* aqueous leaf extract. Journal of Radiation Research and Applied Sciences 2016; 9(1): 1-7.
26. Jadhav K, Dhamecha D, Bhattacharya D and Patil M: Green and eco-friendly synthesis of silver nanoparticles: Characterization, biocompatibility studies and gel formulation for treatment of infections in burns. J of Photochem and Photobiol, B: Biol 2016; 155: 109-15.
27. Abid JP, Wark AW, Brevet PF and Girault HH: Preparation of silver nanoparticles in solution from a silver salt by laser irradiation. Chem Commun 2002; 792-93.
28. Eutis S, Krylova G, Eremenko A, Smirnova N, Schill AW and El-Sayed M: Growth and fragmentation of silver nanoparticles in their synthesis with a FS laser and CW light by photo-sensitization with benzophenone. Photo-chem Photobiol Sci 2005; 4: 154-59.
29. Zhang, Yu JC, Yip HY, Li Q, Xu KW and Kwong AW: Ambient light reduction strategy to synthesize silver nanoparticles and silver-coated TiO2 with enhanced photocatalytic and bactericidal activities. Langmuir 2003; 19: 10372-380.
30. Monga J, Pandit S, Chauhan CS and Sharma M: Cytotoxicity and apoptosis induction in human breast adenocarcinoma MCF-7 cells by (+)-cyanidan-3-ol. Exp. Toxicol. Pathol 2013; 65(7-8): 1091-00.
31. Monks A, Scudiero D, Skehan P, Shoemaker R, Paull K, Vistica D, Hose C, Langley J, Cronise P, Vaigro-Wolff A, Gray-Goodrich M, Campbell H and Mayo J: Feasibility of high fluxanticancer drug screen using a diverse panel of cultured human tumour cell line. Journal of the National Cancer Institute 1991; 83: 757-66.
32. Mosmann T: Rapid colorimetric assay for cellular growth and survival: application to proliferationand cytotoxicity assays. J of Immunol Methods 1983; 65: 55-63.
33. Nagamine MK, Silva TC, Matsuzaki P, Pinello KC, Cogliati B, Pizzo CR, Akisue G, HaraguchiM,Górniak SL, Sinhorini IL, Rao KV, Barbuto JA and Dagli ML: Cytotoxic effects of butanolic extract from *Pfaffia paniculata (*Brazilian ginseng) on cultured human breast cancer cell line MCF-7. Exp Toxicol Pathol 2009; 61: 75-82.
34. Dawidowicz AL and Olszowy M: Mechanism change in estimating of antioxidant activity of phenolic compounds. Talanta 2012; 97: 312-7.
35. Rubinstein LV, Shoemaher RH, Paull KD, Simon RM, Tosmi S, Skehau P, Scudievo DA, Monks A and Boyd MR: Comparison of *in-vitro* anticancer - drug-screening data queerated with terazolium assay-screening assay versus a protein assay against a diverse paued of human tumor cell tissues. JNCI 2003; 82(13): 113-18.
36. Galati G, Teng S, Moridani MY, Chan TS and Brien PJ: Cancer chemoprevention and apoptosis mechanisms induced by dietary polyphenolics. Drug Metabolism and Drug Interaction 2000; 17: 311-49.
37. Vishal G, Thangavel and Sivakumar: Anticancer activity of *Tephrosia purpurea* and *Ficus religiosa* using MCF 7 cell lines. Asian Pacific J of Tropical Med 2011; 526-529.
38. Jadeeswara RK: Anticancer activity of methanolic extract of *Berberis aristata* in MCF-7 human breast cancer cell lines. International Journal of Life Sciences Biotechnology and Pharma Research 2015; 4(1): 31-35.
39. Bama P, Sankaranarayanan S and Bhuvaneswari N: Cytotoxicity effect of alkaloidal compound isolated from the leaf of *Alangium salviifolium* on *in-vitro* MCF-7 cell line. International Journal of Pharmacy and Pharmaceutical Sciences 2012; 4(4): 210-14.
40. Mameneh R, Shafiei M, Aidy A, Karimi E, Badakhsh B and Abbasi N: Toxicity study of silver nanoparticles synthesized from aqueous flower extract of *Scrophulari astriata* on MCF-7 human breast cancer cell line. Phcog Mag 2019; 15: 66-72.
41. Sayed R, Sabry D, Mostafa-Hedeab G and Ali HHM: *In-vitro* characterization and evaluation of silver nanoparticles cytotoxicity on human "liver and breast" cancer cells versus normal melanocytes. The Egyptian Journal of Histology 2019; 42(3): 755-66.

How to cite this article:
Dharmaraj V and Raj JS: Synthesis of silver nanoparticles using leaves and stem bark of *Pterocarpus indicus* and its activity on human breast cancer cell line (MCF-7). Int J Pharm Sci & Res 2021; 12(9): 4807-16. doi: 10.13040/IJPSR.0975-8232.12(9).4807-16.