ASSESSMENT OF ANTICANCER ACTIVITY OF *SOLANUM TORVUM* L. NANOPARTICLES

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Abstract

In recent years, metal nanoparticles have received considerable attention in pharmaceutical applications. These nanoparticles can be synthesized either chemically or biologically. Biological synthesis of nanoparticles is less time consuming, less costly and more eco friendly. In the present study, the green synthesis of selenium nano particles from the *Solanum torvum* fruit extract has been carried out and characterized by UV-Vis spectra, SEM and FTIR analysis. The antimicrobial activity of the synthesized SeNPs against various pathogens and also the cytotoxic activity against MCF-7 breast cancer cell line were determined. *Solanum torvum* fruits (sundaikkai) were collected in November and December 2013 from the local areas of Coimbatore. The functional groups were observed in the sample using FTIR analysis. The SEM image was analyzed to confirm the particle size and appearance of club formed at 93nm. The antimicrobial activity was screened against *E.coli, Bacillus sp., Pseudomonas sp.* and *Klebsella sp.* The maximum activity occurs at 2mM concentration of selenium nanoparticles. Exposure to increasing concentration of SeNPs showed dose-dependent cytotoxicity on MCF-7 cells. These results throw light on the potent anticancer activity of *Solanum torvum* mediated selenium nanoparticles.

Introduction

Anti-cancer agents discovered from plants have played an important role in the treatment of cancer. Compounds derived from plants may not serve directly as drugs, but they provide leads for the development of potential anti-cancer agents. Plant represent an enormous diversity on earth so it was anticipated that plants can provide potential bioactive compounds for the development of leads against various diseases indeed for greater than compounds achievable by synthesis (Khazir *et al.*, 2013).

An alternative solution to western medicine embodied with severe side effects is the uses of medicinal plant preparations to arrest the insidious nature of the disease. Many herbs have been evaluated in clinical studies and are currently being investigated phytochemically to understand their tumouricidal actions against various cancers.

The family Solanaceae represent one of the most economically and medicinally important families of angiosperms. The genus *Solanum* is a hyper-diverse taxon of

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this family. There are about 2000 species of *Solanum* in the world that are mainly distributed in the tropical and sub-tropical areas, with a small number in the temperate areas. About 21 species and one variety in this genus are used as herbal medicines (Hu *et al.*, 1999).

Solanum torvum L. is a small solanaceous shrub, distributed widely in Pakistan, India, Malaya, China, Philippines and tropical America (Nasir, 1985). For many decades, different ethnic groups have used the dried stem and root of this plant for treatment of various ailments. In India, berries of this plant is commonly used in the treatment of various ailments and also used as vegetable.

In recent years, metal nanoparticles have received considerable attention in pharmaceutical applications (Chan and Nie, 1998). These nanoparticles can be synthesized either chemically or biologically. Biological synthesis of nanoparticles is less time consuming, less costly, and more eco friendly.

Selenium (Se) is one of the essential trace elements which play a vital role in the human body by improving the activity of the seleno-enzyme, glutathione peroxidase and preventing free radicals from damaging cells and tissues (Zhang *et al.*, 2004). Se supplementation with low doses seems to be beneficial not only for cancer prevention, but it can positively influence many other functions in an organism by reducing inflammations, heart diseases and regulating the blood pressure (Brozmanova *et al.*, 2010).

In the present study, the green synthesis of selenium nano particles from the *S.torvum* fruit extract has been carried out and characterized by UV-Vis spectra, SEM and FTIR analysis. The antimicrobial activity of the synthesized SeNPs against various pathogens and also the cytotoxic activity against MCF-7 breast cancer cell line were determined.

Materials and methods

Collection of the sample

Solanum torvum fruits (sundaikkai) were collected in November and December 2013 from the local areas of Coimbatore. Fruits were cleaned thoroughly and dried at room temperature (26±2°c) for 5-7 days in the shade. The dried samples were powdered separately using an electrical grinder.

Synthesis of selenium nanoparticles

Selenous acid (H_2SeO_3) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Shade dried *S.torvum* fruits were soaked overnight and crushed finely. For extract preparation the finely crushed *S.torvum* fruits were refluxed for 30 minutes in distilled water. The extract obtained was filtered twice with Whatman paper No.1 and stored at 4°C till further use. Extract (1 mL) was added to solution of 30 mM selenious acid (10 mL) along with 200 µl of 40 mM ascorbic acid which was used as an initiator of reduction reaction. Standard positive control was maintained using 0.2 percent sodium alginate + selenious acid and 200 µl of 40 mM ascorbic acid for the synthesis of selenium nanoparticles while one percent *S.torvum* extract + 200 µl of 40 mM ascorbic acid was used as negative control. The preparations were incubated at room temperature.

At various time intervals, small aliquot (1 ml) of solution was used for the UV–Vis spectroscopy analysis. After 24 h of incubation, the preparation was centrifuged at 10,000 rpm for 30 minutes. The pellet was washed with double-distilled water and then with absolute ethanol three times, this washed ethanol pellet was dried overnight. The red SeNPs were suspended in PBS (pH 7.4) by Ultra sonication and then centrifuged. The powder form of the extract was used for further analysis.

Phytochemical studies

Preliminary phytochemical analysis were carried out according to the standard method of Yadav and Agarwala (2001) for the following chemical compounds such as alkaloids, terpenoids, phenols, tannins, carbohydrates, saponins, flavanoids, quinines, proteins and sterols.

Characterization of selenium nanoparticles

UV-Visible spectroscopy analysis

The bioreduction of the Selenious acid in solution was monitored by periodic sampling of aliquots (1ml) and measuring the UV–Vis spectra of the solution with a Shimadzu 1,700 UV–Vis spectrophotometer at wavelength ranging between 200 and 1,000 nm with a scanning speed of1, 856 nm / min. The readings were recorded at 5, 30, 60, 180, 720 and 1,440 min.

FTIR spectroscopy analysis

For FTIR measurements, the air-dried powder form of the samples was ground with KBr pellets and analyzed on a Thermo Nicolet model 6700 spectrum instrument in the diffuse reflectance mode operating at a resolution of 4 cm^{-1}. To obtain good signal / noise ratio, 512 scans were recorded. The peaks obtained were plotted as percent transmittance in X axis and wave number (cm^{-1}) in Y axis.

Scanning electron microscope(SEM) Analysis

Scanning electron microscope (SEM) images were taken for the analysis of size and shape of SeNPs (Hitachi s-3400N) with resolution of 500 nm operated at 10 kV HV mode and detectors contain secondary electron; semiconductor BSE (Quad type).

Antimicrobial activity

Antibacterial activity of synthesized selenium nanoparticles was determined by well diffusion method. The aqueous extract against *Escherichia coli, Bacillus subtilis, Pseudomonas sp.* and *Klebsella sp.,* was used to find the antimicrobial resistance using Muller Hinton Agar plates. The plate was incubated at 37°C for 24 hours. After that the inhibition of zone was measured.

Anticancer activity

Cell cytotoxic assay

Cytotoxicity was evaluated using MTT assay. Sub confluent monolayer culture of MCF-7 cells was trypsinized and the cells were collected in growth medium. The suspension was centrifuged at 1400 rpm for 5 minutes and the cell pellet was resuspended in growth medium. Viable cells were quantitated using Tryphan Blue Dye Exclusion method. The cells were diluted to 5×10^4 cells/ ml and made up to 20 ml of cell suspension per microtitre plate. Then 200 µl of cell suspension to each wells including control were plated at a cell

density of 1x 10^4 cells/ well and was incubated in the five percent CO_2 incubator for 24 hours to enable them to adhere properly to 96 well polystyrene micro plates. After 24 hours, media was removed. Extracts was prepared at 1 mg/ ml concentration and mixed with fresh medium to achieve the final working concentration (10 to100 µg/ml). Each concentration of extracts was repeated in three wells. Fresh media was added after incubation at 72 hours at 37°C in a humidified incubator, to each well 20 µl of MTT (5mg/ml in PBS) was added and incubated for four hour at 37°C. After this, viability was assessed by the ability of cells to convert the soluble salt of MTT into an insoluble formazan precipitate which was quantitated spectro photometrically following solubilization in DMSO. The absorbance was recorded on a micro titerplate recorder (Bio Rad Co.) at the wavelength of 570 nm and with the reference wavelength at 630 nm. The percent of inhibition of each concentration was calculated by the following formula:

$$\% \text{ of inhibition} = \frac{\text{Dose O.D - Control O.D}}{\text{Control O.D}} \times 100$$

Inhibition concentration (IC_{50}) was evaluated by plotting graph with concentration (µg) of plant extract at X axis and percent of inhibition at Y axis.

Results

Phytochemical analysis

Qualitative chemical examination of aqueous extract of *Solanum torvum* showed the presence of various quantities of alkaloids, carbohydrates, steroids, terpenoids, flavonoids, phenolic compounds, tannins, proteins and amino acids (Table 1).

Table 1. Qualitative Chemical Examination of Aqueous Extracts of *Solanum torvum* Fruits

Phytochemicals	S.No.	Reagents used	Fruit extract
Alkaloids	i.	Mayer's test	+
	ii.	Dragendroff's test	+
	iii.	Hager's test	+
	iv.	Wagner's test	+
Carbohydrates	i.	Molish's test	+
	ii.	Fehling solution	+
	iii.	Benedict's test	+
Steroids and terpenoids	i.	Lieberman's and Burchard's test	+
	ii.	Salkowski's test	+
Flavonoids	i.	Ferric chloride test	+
	ii.	Shinod's test	+
Phenolic compounds and tannins	i.	5% ferric chloride	+
	ii.	10% lead acetate	+
Saponins	i.	Foam test	-
Proteins and amino acids	i.	Ninhydrin test	+

+ Presence - Absence

Characterization of selenium nanoparticles

UV-Vis spectroscopy analysis

Biosynthesis of SeNPs from Selenious acid was confirmed by UV–Vis spectra studies, due to color change from colorless selenious acid to ruby red color (SeNPs), having absorption maximum (kmax) at 200–400 nm. This color change may be due to the surface plasma resonance (SPR) with a broad peak, and this peak intensity of color change increased with the time from 5 to 1,440 minutes. The small peaks observed in the UV region may be due to the small organic molecules present in reaction mixture (Figure 1).

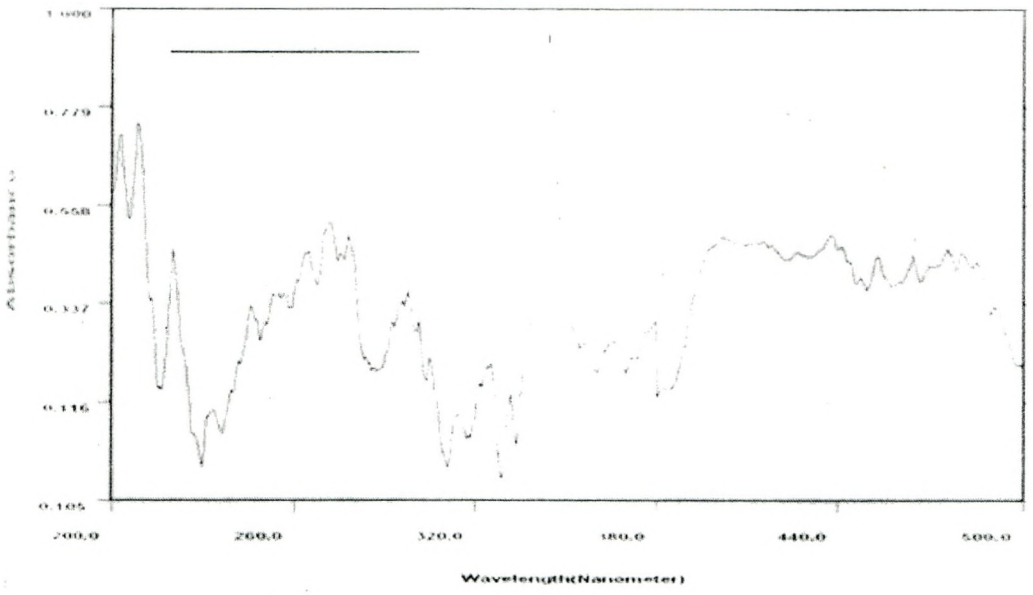

Figure 1. UV-Vis spectra of SeNPs synthesized by *S.torvum* fruit extract

FTIR analysis

FTIR spectrum of *S.torvum* fruit extract showed the presence of various functional groups present in different combination of extracts. FTIR spectrum was recorded from 400- to 4,000 cm^{-1} wavelength region. Representative spectra at 24 hrs manifest major IR bands at 3480, 3342, 3231, 2115 cm^{-1} and minor bands at 1635, 685, 655 cm^{-1} (Figure 2).

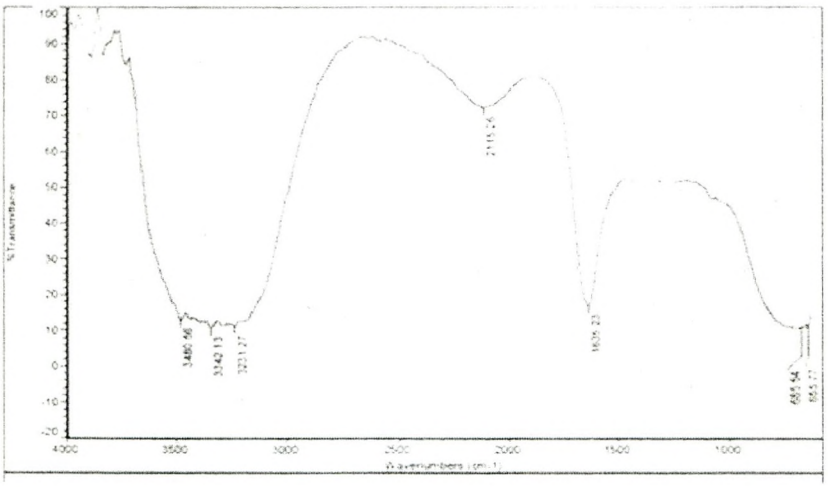

Figure 2. FTIR spectra of SeNPs synthesized by *S.torvum* fruit extract

SEM analysis

The scanning electron microscope (SEM) that images the sample surface by scanning it with a high energy beam of electrons. The synthesized nanoparticles showed below 1µm size. They were oval in shape with smooth surface. The small particles are appearing at 93nm followed to large particle synthesis at 120 nm (Figure 3). The SEM image shows cluster of nanoparticles at two different size of 24 hours incubation time of product.

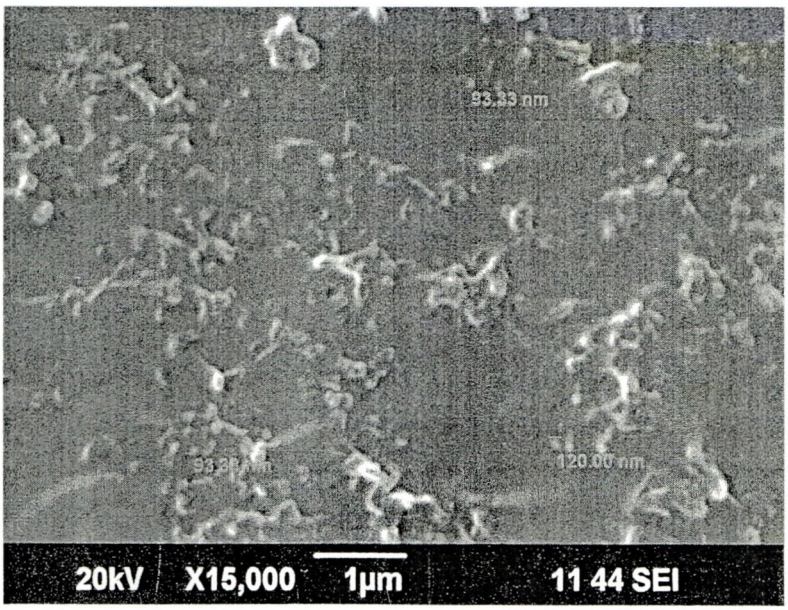

Figure 3. SEM image of SeNPs synthesized using *S.torvum* fruit extract

Antimicrobial activity

The pathogenic microorganisms subjected in the present study were *Bacillus sp., E.coli, Pseudomonas sp.* and *Klebsella sp.* using agar well diffusion method. The mean of three replicates of zone of inhibition (mm) around well with *S.torvum* mediated selenium nanoparticles is presented in the Table 2.

Table 2. Zone of Inhibition (mm) of *S.torvum* Mediated Selenium Nanoparticles

Microorganisms	Aqueousextract	Se.NP	1mM Se.NP extract	2mM Se.NP extract
Bacillus sp.	2mm	2mm	5mm	8mm
E.coli	Nil	2mm	4mm	6mm
Pseudomonas sp.	Nil	2mm	4mm	6mm
Klebsella sp.	4mm	5mm	5mm	8mm

The number of bacterial colonies grown on agar plates as a function of different concentrations of selenium nanoparticles was presented in the Figure 4. The bacterial colonies are found to be decreased gradually as the concentration of nanoparticles increased.

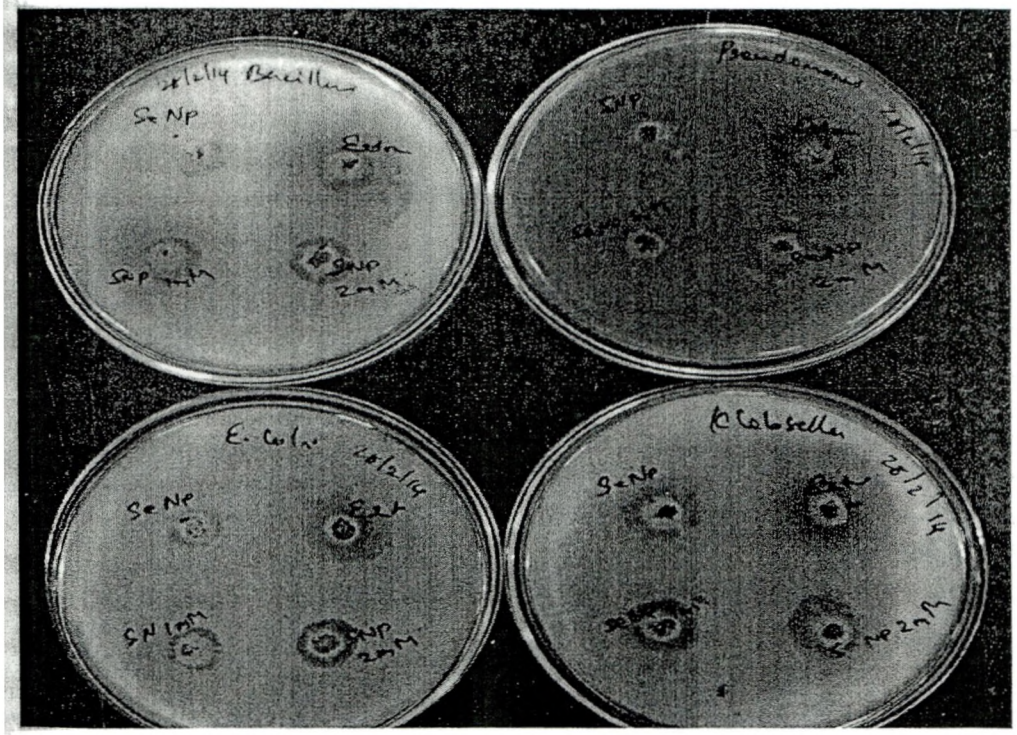

Figure 4. Antimicrobial activity *of S.torvum* mediated SeNPs

Anticancer activity

The antiproliferative activity of the aqueous extract of *S. torvum* showed a dose dependent MTT reduction in breast cancer cell lines. The 50 percent inhibitory concentration was found to be 66.26 µg/ml (Table 3).

Table 3. Anti Proliferative Activity of *S. torvum* on MCF-7 Cell Lines

Plant extract	Conc.(µg/ml)	% inhibition	IC 50(µg/ml)	R^2
Solanum torvum	18.75	1.58	66.26 µg/ml	0.94
	37.5	26.5		
	75.00	49.74		
	150.00	92.70		
	300.00	100		

Discussion

The medicinal value of plants lies in the chemical substances that produce a definite physiological action on the human body. In the present study phytochemical screening was performed in aqueous extract of *Solanum torvum* fruits which showed the presence of alkaloids, flavonoids, amino acids, proteins, carbohydrate and cardiac glycosides.

With the recent development of nanotechnology, nano-selenium has attracted widespread attention because nanometer particulates exhibit novel characteristics such as large surface area, high surface activity, high catalytic efficiency, strong adsorbing ability and low toxicity. It has been reported that nano-Se possesses comparable efficiency to selenite and Se-methyl selenocysteine in up regulating seleno enzymes but with dramatically decreased toxicity (Zeng *et al.*, 2008).

Formations of stable seleniu nanoparticles using the fruit extract of *S.torvum* in aqueous solution are confirmed using UV Vis spectral analysis. Characteristic absorption bands are observed at 344 nm for selenium nanoparticles. It has been postulated that plant extract containing phenol and flavonol derivatives may act as reducing agents and nanoparticle stabilizer.

FTIR analysis of synthesized selenium nanoparticles free from proteins and water soluble compounds was done in this direction. The analysis of IR spectra gives an idea about biomolecules bearing different functionalities which are present underlying system.

The minor band 655cm^{-1} appears to the fragmentation of O-CO show oxidioximethyl and carboxyl radical. For another minor band 685cm^{-1} is C-Cl group of 1-chloro-1, 3-butadiyne. However, there is adequate evidence for the presence of

surface bound proteins, the conclusion regarding the presence of the specific compound cannot be made unless the individual component is isolated and identified.

Furthermore, an interfacial interaction of these biomolecules with core particles is the matter of investigation. The absorption frequency observed at 1635 cm^{-1} indicates an HAOAH stretch suggesting water sorption to the mineral surface of hydroxyl ion (OH). The major band of 2115cm^{-1} at Si—H of hydridosilicate (1-), followed to 3231cm^{-1} group of Ammonia anhydrous presence. The sharp band at 3342cm^{-1} is a nano structure most likely due to the fact that the hydroxyl groups are hydrogen bonded intra molecularly, as well as inter molecularly with other OH groups and with other carboxyl groups. The -OH group in an alcohol is replaced by a halogen such as chlorine or bromine may appear at final major band of 3480cm^{-1}.

This supports the strong binding of -C O functionality from medium, to the core particles. A broad intense band at 3400 cm^{-1} in the spectra can be assigned to the N-H stretching frequency arising from the peptide linkages present in the proteins of the extract. The shoulders around the band can be identified as the overtone of the amide-II band and the stretching frequency of the O-H band, possibly arising from the carbohydrates and/or proteins present in the sample. The flattening of the shoulders indicates decrease in the concentration of the peptide linkages in the solution. The spectra also exhibit broad asymmetric band at 3400 cm^{-1} that can be assigned to the N-H stretching band in the free amino groups of selenium nanoparticles. The SEM image was analyzed to confirm the particle size and appearance of club formed at 93 nm.

Selenium compounds reported to have antimicrobial activities and find applications in pharmaceutical industries, for example, use of selenium sulfide in fungicides and antidandruff shampoos manufacturing (Lenz and Lens, 2009).

Currently, the increase of bacterial resistance to antimicrobial agents poses a serious problem in the treatment of infectious diseases as well as in epidemiological practice. Increasingly, new bacterial strains have emerged with dangerous levels of resistance, including both of Gram-positive and Gram-negative bacteria. Dealing with bacterial resistance will require precautions that lead to prevention of the emergence and spreading of multiresistant bacterial strains, and the development of new antimicrobial substances (Panacek *et al.*, 2006).

In the present study, antimicrobial activity of SeNPs was screened against *E.coli, Bacillus sp., Pseudomonas sp.* and *Klebsella sp.* The maximum activity occurs at 2mM concentration of selenium nanoparticles. These results demonstrate the ability of the *S. torvum* on synthesizing selenium nanoparticles and their antimicrobial activity represent a significant advancement. in the nanomaterial with realistic implications. The green chemistry

approach addressed in the present work on the synthesis of selenium nanoparticles is simple, cost effective and the resultant nanoparticles are highly stable and reproducible.

Like other chemotherapeutics, the effective cytotoxicity of nanomaterial based therapies usually requires a fairly high level of accumulation within the cancer cells. Although nano materials tend to accumulate in cancer cells through a passive targeting process and often serve as "nanocarriers" for chemotherapeutics, this passive strategy has limitations due to its random delivery mode. Studies have shown that enhancement of tumor accumulation of nanoparticles can be achieved by increasing the cellular uptake of functionalized nanoparticles. During applications of cancer nanotechnology, active targeting of nanomaterials is usually achieved by conjugation of a targeting component to the surface that provides preferential accumulation of nanomaterials in the tumor-bearing organ, in the tumor itself, individual cancer cells, or intracellular organelles inside cancer cells.

Several mechanisms have been postulated to elucidate the anticancer action of Se, including induction of apoptosis, effects on the cell cycle distribution, inhibition of angiogenesis, protection against oxidative stress, detoxification of carcinogens, stimulation of the immune system, modulation of thioredoxin reductase activity and maintenance of cell redox balance.

In the present study, it is found that the cytotoxic effect of SeNPs of *S.torvum* against breast cancer cell lines. The level of concentration ranging from18.75 µg/ml to 300 µg/ml of selenium NP synthesized *Solanum torvum* was used. The minimum inhibitory concentration (IC50) of SeNPs on MCF-7 cells was obtained at 66.26µg/mL at 24 hours. Exposure to increasing concentration of SeNPs shows dose-dependent cytotoxicity on MCF-7 cells. The present study correlates with the results of an earlier study (Sowemimo *et al.*, 2009) where *Spanim* leaves showed the highest cytotoxic activity against MCF-7 cell line. Cannonball leaves have also been reported to have antioxidant activity and this may have a role to play in the observed activity in the cancer cell lines as antioxidants play a complex role in cancer prevention (Martinez *et al.*, 2012).

More clinical trials should be conducted to support the therapeutic use of *Solanum torvum* selenium nanoparticles. It is also important to recognize that *Solanum torvum* mediated nanoparticle extracts may be effective not only when used singly, but may actually have a modulating effect when given in combination with other herbs or drugs.

Conclusion

In the present study aqueous extract of *Solanum torvum* was used to synthesize the selenium nanoparticles which are characterized to find out the antimicrobial and anticancer activity. SeNPs were characterized by UV spectrophotometer in

order to find out the highest peak at 344nm. The functional groups were observed in the sample using FTIR analysis. The SEM image was analyzed to confirm the particle size and appearance of club formed at 93nm. The antimicrobial activity was screened against *E.coli, Bacillus sp., Pseudomonas sp.* and *Klebsella sp.* The maximum activity occurs at 2mM concentration of selenium nanoparticles. Further characterization of SeNP's against breast cancer cell lines gave a significant antiproliferative activity against the cancer cells. The minimum inhibitory concentration (IC50) was found to be 66.26µg / mL at 24 hours. Exposure to increasing concentration of SeNPs showed dose-dependent cytotoxicity on MCF-7 cells. However, further investigations were needed to identify the scaling-up usage of this extract on metallic nanoparticle synthesis and its applications on anticancer therapy.

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