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Original Research Article

Sapindus emarginatus extract embedded with gold nanoparticles: an antiproliferative agent against MCF7 breast cancer cell line

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ABSTRACT

There are numerous studies reported on the usage of the Sapindus emarginatus (SE) fruit in cancer and other treatments in the past few years. In this study, crude SE fruit extract was prepared and it was further used to synthesis gold nanoparticles (Au Nps). The synthesized Au Nps were left embedded in the SE fruit extract. The Au Nps embedded in the SE fruit extract (SE-Au Nps) were characterized using UV Vis spectroscopy, Centrifugal particle size analyzer (CPS), Scanning Electron Microscope (SEM) and Fourier Transform Infrared Spectroscopy (FTIR). MTT assay was carried out for both SE fruit extract and SE-Au Nps on MCF7 breast cancer cell line and compared. The UV- Vis Absorbance for the SE-Au Nps was obtained at 543nm. The centrifugal particle size analysis of the Au Nps embedded in SE fruit extract showed the size of the nanoparticles to be widely varying with higher fraction of particles between the size ranges of 15 to 20nm. The morphology of the Au Nps embedded in SE fruit extract was observed using SEM. The presence of Au Nps in SE fruit extract was confirmed using FTIR. The results of the MTT assay on MCF7 breast cancer cell line proved that the %cell viability was less for SE-Au Nps than that of the SE fruit extract alone. Thus the antiproliferative activity of the SE fruit extract was significantly enhanced by embedding it with Au Nps and it can be effectively used in therapeutic applications after further studies.

Keywords: *Gold nanoparticles; Sapindus emarginatus; Antiproliferative; MCF7.*

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1. Introduction

Cancer is the abnormal growth of cells with uncontrolled division resulting in increased number of cells (Manoharan *et al*., 2012). Cancer is observed as the most dangerous class of disease categorized by uncontrolled cell growth (Chow 2010; Suriamoorthy *et al*., 2010). There is a marginal increase in cancer cases in the last few years, and most of the time, it ends up with taking life (Dite *et al*., 2010; Parveen and Sahoo, 2010). Breast cancer is a complex and heterogeneous disease (Deborah L Holliday and Valerie Spiers, 2011). In world breast cancer represents 9% of the global cancer burden and is the third most common tumour. Human breast cancer MCF7 cells represent one of the most widely used experimental models for in vitro studies on breast carcinoma (Manar and Awatif, 2012).

A considerable part of the current knowledge on breast carcinomas is based on in vivo and in vitro studies performed with cell lines derived from breast cancer. In reference to the treatments available for cancer, the characteristics of the cancer determine the treatment, which may include surgery, medications (hormonal therapy and chemotherapy), radiotherapy and immunotherapy. There are currently three main groups of medications for breast cancer such as hormone blocking agents (Tamoxifen, Anastrozole or Letrozole), chemotherapy (cyclophosphamide, methotrexate and fluorouracil) and monoclonal antibodies (Trastuzumab). Radiotherapy is given after surgery to the region of the tumor bed and regional lymph nodes, to destroy microscopic tumor cells that may have escaped surgery. Radiation can reduce the risk of recurrence by 50-60% (Florescu *et al*., 2011). Most chemotherapy medications work by destroying fast-growing and/or fast-replicating cancer cells, either by causing DNA damage upon replication or by other mechanisms. Along with the medications chemotherapy and radiation can also affect healthy cells. Damage to the heart muscle is the

most dangerous complication of doxorubicin, for example. Trastuzumab is very expensive and its use may cause serious side effects (approximately 2% of patients who receive it suffer from significant heart damage). Due to growing resistance and side effects to these therapies search for new therapeutics for breast cancer has become essential. Scientific interest in medicinal plant has bloomed in recent times due to increased efficiency of new plant derived drugs and wide spread concerns about the side effects of modern medicine (Parekh *et al*., 2007).

Sapindus emarginatus Vahl. found in South India is commonly known as soap nut tree. The tree species is inadequately distributed in diverse geographical provinces like Gangetic plains, Western Ghats and Deccan Plateau in India (Mahar *et al*., 2011). The genus Sapindus possesses tremendous medicinal value. Since past, it is used as emetic, tonic, astringent, anthelmintic, for asthma, colic, diarrhea, cholera, tubercular glands and paralysis of limbs. Methanolic extract of fruit of Sapindus emarginatus (SE) found to produce CNS depressant activity (Chattopadhyay *et al*., 2003). The fruits are usually used for hair problems and also in preparation of shampoos. Traditionally SE is used as anti-inflammatory and antipruritic medicine. The seed is an intoxicant and the fruit rind has oxytropic action. Its powder is used as nasal insufflations (Nair *et al*., 2005). Saponins isolated from different plants and animals have been shown to specifically inhibit the growth of cancer cells in vitro (Kuznetzova *et al*. 1982; Rao & Sung, 1995; Konoshima *et al*., 1998; Marino *et al*., 1998; Mimaki *et al*., 1998; Podolak *et al*., 1998). The saponins from SE fruit extract found to have significant antihyperlipidemic activity (Srikanth Jeyabalan and Muralidharan Palayan, 2009).

The combined application of saponins with other antitumor compounds may increase cytotoxic activity of the latter, which is an interesting new possibility in cancer treatment research (Hebestreit and Melzig, 2003). The noble metal nanoparticles like gold nanoparticles represent smart and promising candidates in the drug delivery applications due to their unique dimensions, tunable functionalities on the surface, and controlled drug release (Datar and Richard, 2010). Another essential aspect while working with AuNP in bio-applications is safety and biocompatibility (AuNP is already approved by the US Food and Drug Administration.) Biologically synthesized and functionalized, AuNP provide many desirable attributes for use as carriers in drug delivery systems as the functionalized AuNP core is essentially inert and nontoxic reported in recent studies (Han *et al*., 2007). The labeling of AuNPs with biological ligands to specifically bind to desired cancer cells increases the effectiveness of thermal energy transfer to cancer cells without harming non-cancerous cells (Jain, *et al*., 2007). After cellular uptake, The Au Nps can act as tiny, precise and powerful heaters (thermal scalpels) to kill cancer and they are capable of inducing apoptosis in B-chronic lymphocytic leukemia (Mukherjee *et al*, 2007).

Ashwani Kumar *et al*. (2013) has found that Gold nanoparticles embedded in *Rubia cordifolia* (RC) matrix significantly enhanced anti-inflammatory characteristics by inhibiting nitric oxide release. It was reported in terms of inhibitory concentration for 50% inhibition compared to either RC extract or AuNPs. Hence, in this work the SE fruit extract was embedded with gold nanoparticles with the vision of increasing its antiproliferative activity.

2. Materials and Method

2.1 Preparation of Plant Extract

Fresh fruits of SE were collected from the nursery of Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore, Tamilnadu. Collected SE fruits were dried under shade, mechanically powdered and stored in an airtight container. Dried and powdered SE fruits were extracted with 95% methanol in a Soxhlet extractor. The methanolic extract was concentrated to give a dark brown residue which was partitioned between water-n-Butanol (1:1). The n-butanolic layer was evaporated to give the crude saponin fraction as a brown residue (Uma Prawat *et al*., 1989).

2.2 Synthesis of Gold Nanoparticles (Au NPs) Embedded in Sefruit Extract (SE-AU NPs)

A solution of 50 ml, 0.001M (0.39382 mg/ml) Gold (III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$) and 50 ml SE fruit extract, diluted in 50 ml of distilled water, was added together drop by drop and stirred on a magnetic stirrer. The solution was ultrasonicated at a high frequency of 10 KHz for 3h and then maintained at a stationary position for 2h at room temperature.

2.3 Characterization of SE-AU NPs

SE-Au Nps were characterized by UV-visible Absorption spectroscopy, Centrifugal Particle size analyzer (CPS), Fourier transform infrared spectroscopy (FTIR) and Scanning electron microscope (SEM).

2.4 UV-visible absorption spectroscopy studies

UV-Vis absorption spectra have been proved to be quite sensitive to the formation of gold colloids because gold nanoparticles exhibit an intense absorption peak around 540nm due to the surface plasmon (it describes the collective excitation of conduction electrons in a metal) excitation. The sample was analyzed using UV-9000S Spectrophotometer (Lark, India). Distilled water was used as a blank.

CPS:

The gold nanoparticles embedded in the plant extract were isolated by centrifugation. Diluted suspensions of sucrose, on the order of 0.01 – 1.0 [wt%], were prepared. The sugar solution and our sample solutions were injected in disc centrifuge. Nanoparticle size was analyzed by injection of 350µl of sample into CPS operating at a speed of 20,000 rpm. All analyses were run against a known calibration standard and the particles in the solution were analyzed by size distribution graph.

SEM:

Morphology of the synthesized Au Nps in the SE fruit extract was analyzed using SEM. Few drops of the SE fruit extract embedded with the Au Nps was spread on a cover slip and dried. Then the sample on the cover slip was coated with gold in a sputter coating unit for few minutes.

FTIR:

The samples were completely dissolved in respective solvents, non-sticky and the pH was found to be less than 8 which are necessary conditions to analyze the liquid samples. The sample analysis was carried out using FTIR (Brucker – Tensor 27) in ATR mode with a range from 500 to 4000 cm^{-1}. The liquid samples are directly placed onto the ZnSe crystal to obtain the spectrum.

3. Cell Proliferation Assay on Mcf7 Cell Line

3.1 Chemicals and Reagents

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) Invitrogen, USA. Acridine orange were obtained from Sigma, USA. All other fine chemicals were obtained from Sigma–Aldrich, St. Louis.

3.2 Cell Culture

MCF7 cells obtained from NCCS (National Centre For Cell Science, Pune) were cultured in Rose well Park Memorial Institute medium (RPMI), supplemented with 10% fetal bovine serum, penicillin/streptomycin (250 U/ml), gentamycin (100 µg/ml) and amphotericin B (1mg/ml) were obtained from Sigma Chemicals, MO, USA. All cell cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2. Cells were allowed to grow to confluence over 24 h before use.

4. Cell Growth Inhibition Studies by Mtt Assay

Cell viability was measured with the conventional MTT reduction assay, as described previously with slight modification. Briefly, MCF7 cells were seeded at a density of 5×10^3 cells/well in 96-well plates for 24 h, in 200 µl of RPMI with 10% FBS. Then culture supernatant was removed and RPMI containing various concentrations (0.11–100 µg/ml) of test compound was added and incubated for 48 h. After treatment cells were incubated with MTT (10 µl, 5 mg/ml) at 37 °C for 4 h and then with DMSO at room temperature for 1 h. The plates were read at 595 nm on a scanning multi-well spectrophotometer. Data represented the mean values for six independent experiments. (Evelyn *et al*.,2012). Cell viability (%) = (Mean OD/Control OD) × 100.

5. Results and Discussion

5.1 Preparation of Se Fruit Extract

Crude extract of SE fruits was obtained as brown coloured mixture from the Soxhlet apparatus. The mixture was separated into upper phase (butanol and crude saponin) and lower phase. The upper phase was evaporated and the crude saponin obtained was brown in colour with a colourless lower phase.

5.2 Synthesis of SE-AU NP

The change in the brown color of SE fruit extract into purple color after its treatment with $HAuCl_4 \cdot 3H_2O$ solution clearly indicated the formation of Au Nps. The color change was observed within 1h in room temperature. Jannathul Firdhouse and Lalitha (2014) have earlier reported the similar color change as the indicator for the formation of Au Nps. The reduction of metal ions was roughly monitored by visual inspection as described earlier by Fang *et al*., (2005). The process performed simply at room temperature is comparatively free of toxic chemical hazards.

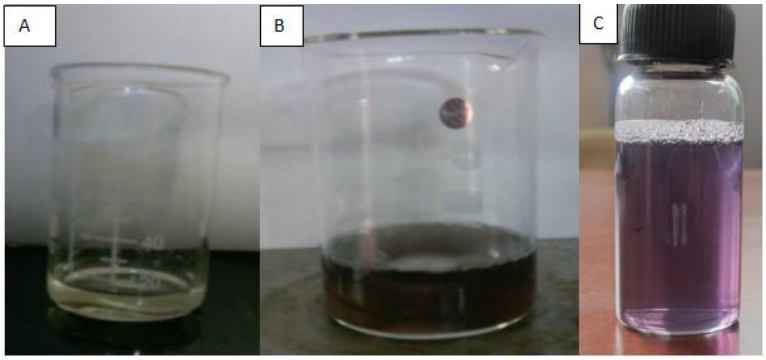

Figure 1. Synthesized AuNp. (A) $HAuCl_4 \cdot 3H_2O$; (B) Plant Extract+ $HAuCl_4 \cdot 3H_2O$; (C) SE-Au NP

5.3 Characterization of Se Au Nps:

Uv Visible Spectroscopy

The UV-Vis Absorbance for the SE-Au Nps was obtained at 543 nm and it is due to the excitation of surface plasmon vibrations in the gold nanoparticles. The spectra are consistent with previous experimental results. Parida *et al*. (2011) reported the synthesis of gold nanoparticles using Allium cepa extract as the reducing agent and the absorption peak was broad and found at 540 nm which might be due to polydispersity nature of the nanoparticles.

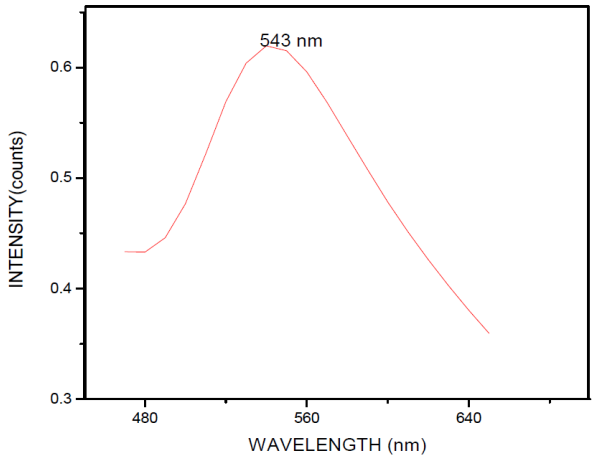

Figure 2; Uv-Visible spectra for SE-Au NPs

CPS

The particle size analyzer (**Figure 3**) showed various diameter ranges of nanoparticles. The diameter versus % fraction was plotted. The size of the Au-Nps embedded in SE fruit extract was found to be widely varying with higher fraction of particles between the size ranges of 15 to 20 nm.

The particle size analyzer displayed a total weight of injected gold sample as 57.26 µg. In the injected sample 20 to 15.9 nm diameter of particles are present in high level whereas the 15.9 to 12.7 nm sized particles were in minimum level. This variation may be due to the aggregation of the nanoparticles in the extract.

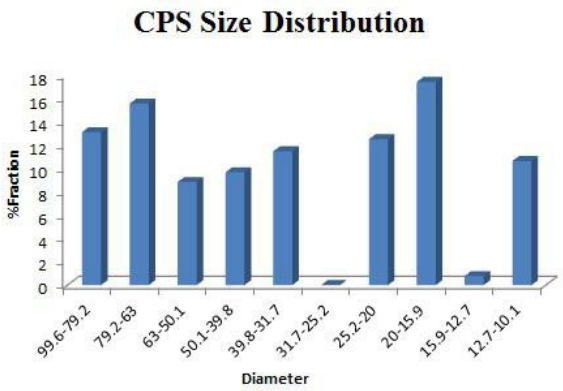

Figure 3; Particle size distribution of Au NPs

SEM:

The SEM image (**Figure 4**) of SE-Au Nps showed the Au Nps distributed over the extract. The Au Nps embedded in the SE fruit extract were found to be aggregated and was not clear. The result obtained was similar to that of Paz Elia *et al.* (2014) who observed the morphology of Au Nps using plant extracts as reducing agents.

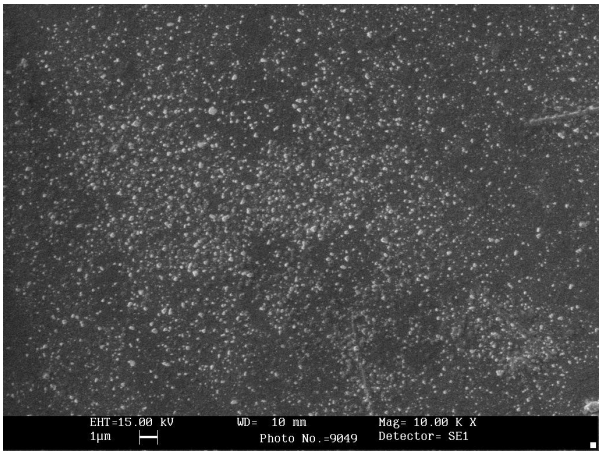

Figure 4. SEM image of SE-Au Nps

FTIR

FTIR spectrum of methanolic fruit extract of SE showed characteristic peaks for several functional groups like hydroxyl group in the range 3500 to 3000, C-O-C in the range of 1500 to 1000 and C-Br (alkyl) in the range of 1000 to 500. For SE-AuNPs the peak in the range of 3500 to 3000 indicates the presence of -NH_2 (amino) group, C-H group at 2119 and carbonyl group C=O in the range of 2000 to 2500. By comparing the spectrum of SE-Au Nps with the spectrum of SE fruit extract and the standard spectrum of gold nanoparticles (Syed Baker & Sreedharamurthy Sathish, 2015), it is clear that the spectra of SE embedded with gold nanoparticles indicates the presence of both the extract and the gold nanoparticle in it.

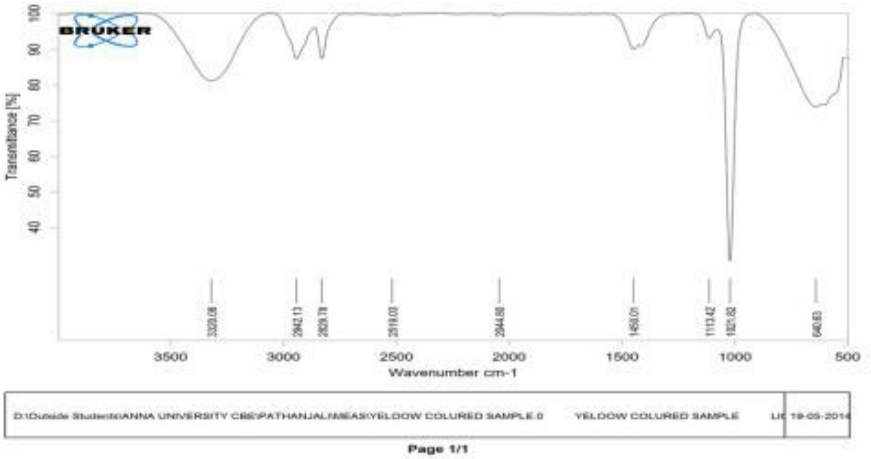

Figure 5. FTIR of SE Fruit Extract

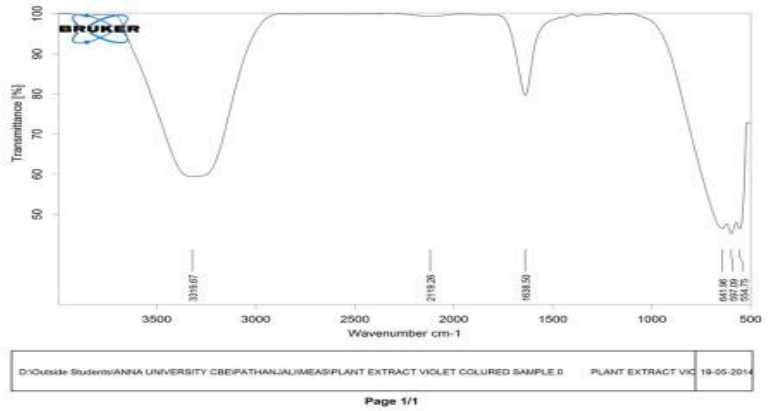

Figure 6. FTIR of SE-Au NPs

6. Cell Proliferative Study-Mtt Assay

The antiproliferative activity of SE fruit extract and SE-Au Nps under in vitro conditions were examined on cell proliferation by the MTT assay. MCF-7 cells were exposed to the two samples at varying concentrations for 48 h and cytotoxicity was determined using MTT assays. MTT results have shown that as the concentration of the samples increase, increased cytotoxicity was observed in a dose-dependent manner. In MTT assay cell viability was significantly reduced to 47, 60 and 81.9% for the concentrations of 100, 10 and 1 µg respectively for SE-Au Nps and 69.9, 85.76 and 90% for 100, 10 and 1 µg respectively for crude SE fruit extract (Figure 7). The SE-AU Nps was found to be more effective compared to the crude SE fruit extract alone which indicates that Au Nps increases the cytotoxicity and has great potential as conjugate with the fruit extract and can be effectively used as anticancer agent.

Concentration	Cell viability (%)	
	Sample A	Sample B
100 µg	47.38003	69.93933
10 µg	60.28682	85.76944
1 µg	81.9636	90.12686
100 ng	98.78654	95.58742
1 ng	97.90403	98.67623

Table 1. MTT assay for cell viability

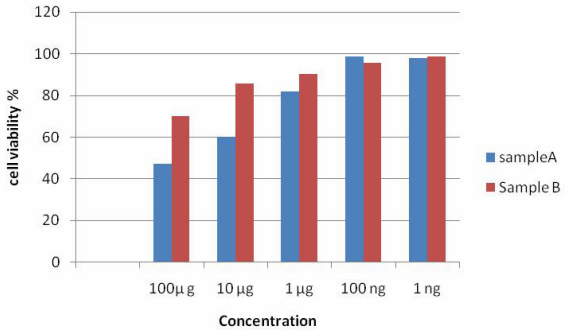

Figure 7. Cell viability determined by MTT assay. Sample A: SE-Au Nps. Sample B: SE fruit extract.

7. Conclusion

The SE fruit extract was prepared using the soxhlet apparatus. The SE fruit extract was used to synthesize the gold nanoparticles. The synthesized gold nanoparticles embedded in the SE fruit extract (SE-Au Nps) were confirmed by its absorbance at 543 nm using the UV Vis spectrophotometer. The size of the Au Nps was found to be widely varying with higher fraction of particles between the size ranges of 15 to 20 nm. This may be due to the agglomeration of the particles. Morphology of the SE-Au Nps was observed using SEM. The FTIR analysis of the SE-Au Nps confirmed the presence of gold nanoparticles in the extract. MTT assay was carried out for both SE fruit extract and SE-Au Nps on MCF7 breast cancer cell line and compared. The results of the MTT assay on MCF7 breast cancer cell line proved that the %cell viability was less for SE-Au Nps than that of the SE fruit extract alone. Thus the antiproliferative activity of the SE fruit extract was significantly enhanced by embedding it with Au Nps and it can be effectively used in therapeutic applications after further studies. The SE-Au Nps can be further taken to in vivo studies and then to clinical applications.

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