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In vitro cytotoxicity of *Dioscorea oppositifolia* L. against breast cancer cell line MCF-7

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Abstract
Green synthesized silver nanoparticles from Diosgenin, an active principle of *Dioscorea* is a steroidal saponin has been characterized by UV-Vis spectroscopy, SEM, TEM, DLS and FTIR. Diosgenin act as a reducing agent converts silver ions to AgNPs in a rapid and eco-friendly manner. The UV-Vis spectra of Diosgenin samples depicts surface plasmon resonance peak at 410-431 nm. TEM image of the sample shows well-dispersed silver nanoparticles with an average particle size of 13 nm. The three dimentional structure of silver nanoparticles was seen through SEM analysis. Diosgenin mediated silver nanoparticles was found to be rod shaped and DLS studies showed approximate size of nanoparticles and their zeta potential value was proved by their high stability nature. FTIR showed functional groups and the stretch of bonds which responds to nanoparticles synthesis were elucidated. Breast cancer is the topmost cancer in women both in the developed and the developing world. The incidence of breast cancer is increasing in the developing world Progress. According to WHO Report, issued in August 2012, provides an update with the latest data on breast cancer incidence and mortality. Adequate therapy is needed for breast cancer that, although not amenable to early detection and treatment, Nanotechnology has high potential for breast cancer treatment. *In vitro* cytotoxicity activity of Diosgenin and its nanoparticles against most prevailing dangerous life threatening disease in women, breast cancer using MCF-7 Cell line. Diosgenin has shown 49% inhibition against breast cancer cell line whereas diosgenin mediated silver nanoparticles has shown 100% inhibition against MCF-7 breast cancer line. This is the first report of synthesizing silver nanoparticles from Diosgenin, *Dioscorea oppositifolia* plant active principle which is a triterpenoids steroidal saponin and are used as cortico-steroidal drug and its efficacy was successfully analysed for breast cancer treatment.

Keywords: breast cancer, antioxidant parameters, Ao/Eb staining

Introduction
Yams belong to the genus *Dioscorea* of the family Dioscoreaceae. Among 600 species, *Dioscorea oppositifolia L., D. alata L., D. cayenensis Lam., D. rotundata Poir., D. trifida L., D. esculenta Burkill, and D. bulbifera L.*, have the significant medicinal and economic importance. Among the *Dioscorea, D. oppositifolia* L. has more nutritional and economical value. *D. oppositifolia* L. is rich in nutrients including proteins (3.59 % to 8.93 %), amino acids (2.31 % to 7.26 %), starches (43.7 %), sugars (3.39 %), vitamins, and amylases**.** In addition to that, two active constituents are present in *D. oppositifolia* L. tubers, such as Diosgenin and allantoin (Fu *et al*., 2006) [1]. Steroidal saponin, particularly diosgenin and its glycoside, are the typical bioactive components for most plants of the family Dioscoreaceae (Yang and Lin, 2008) [18].

Traditionally, the tuber is used to treat inflammation, joint pain, diabetes, infections, dysmenorrhea, rheumatism, and arthritis. *Dioscorea* tuber is known to biosynthesize steroidal compounds like saponin dioscin which gets converted to sapogenin diosgenin on hydrolysis The discovery of diosgenin (natural steroid) in the tubers has made it one of the most researched and studied herbal product. Many health benefits are associated with diosgenin, like prevention against cardiovascular disease, cancer and contraception. Diosgenin is used as a starting material for the synthesis of steroidal drugs, particularly for the partial synthesis of oral contraceptives, sex hormones and other steroids, since it exhibits estrogenic activity. Diosgenin has received considerable attention because of the variety of their promising pharmaceutical properties like lowering serum cholesterol level. The consumption of diosgenin has been reported to cure skin diseases, tuberculosis, diabetes, jaundice, hypertension, emotional instability, mental illness and has positive actions on stress and inflammatory conditions. Diosgenin has been used in traditional Chinese medicine for treatment of urethral and renal infections. (Heena and Lele, 2012) [4].

A new Indian source for diosgenin is from *Dioscorea*, which is used to induce apoptosis in cancer cells and to reduce high blood pressure. Over the past decade, a series of preclinical and mechanistic studies have been conducted worldwide to understand the role of diosgenin as a chemo preventive agent against several cancers (Raju and Mehta, 2009) [14].

Diosgenin have been found to exerts its anticancer effects against a wide variety of tumor cells, including breast cancer, colorectal cancer, osteosarcoma, and leukaemia (Srinivasan, 2009). The antitumor effects of diosgenin have been demonstrated to be mediated through activation of p53, immune-modulation, cell cycle arrest, modulation of caspase-3 activity, and induction of TRAIL death receptor DR5 (Lepage *et al*., 2011) [8]. Diosgenin inhibited proliferation and induced apoptosis in HepG2 cells by

inhibiting signal transducer and activator of transcription (STAT3) signaling pathway (Kim *et al*., 2007) [7]. Diosgenin has been shown to target multiple pathways of tumorigenesis; including proliferation, apoptosis, angiogenesis, invasion, and tumor-induced immuno suppression in various tumor cells and *in vivo* cancer models (Raju and Mehta, 2009) [14].

The search for high-producing cell lines coupled to recent developments in immobilized cultures and the use of extraction procedures, which convert furostanol saponin to spirostanes such as diosgenin, should prove useful in increasing productivity in the years to come. There is a huge demand of diosgenin in the pharmaceutical industry for the growing population. In the light of the above information, the present work is an attempt to reveal many intricating objectives related with phytodrug discovery and therapeutic potentials in *Dioscorea oppositifolia* to produce environmentally safe approach.

Materials and Methods

Antitumor Efficacy (MTT assay

In vitro cytotoxicity assay) (Monks *et al*., 1991) The human breast cancer cell line (MCF 7) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium (EMEM) containing 10% fetal bovine serum (FBS). All cells were maintained at 37°C, 5% CO_2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week. The human breast cancer cell line (MCF 7) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37^0C, 5% CO2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

Breast cancer cell line Treatment

The monolayer cells were detached with trypsin-ethylene di amine tetra acetic acid (EDTA) to make single cell suspensions and viable cells were counted using a haemocytometer and diluted with medium containing 5% FBS to give final density of $1x10^5$ cells/ml. One hundred micro litres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^0C, 5% CO_2, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the test samples. Samples S1, S3 and S5 were dissolved in neat di methyl sulfoxide (DMSO). An aliquot of the sample solutions were diluted to twice the desired final maximum test concentration with serum free medium. Additional four serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 h at 37^0C, 5% CO_2, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

Apoptotic activity

AO/EB Staining

Acridine orange is a vital dye and will stain both live and dead cells. Ethidium bromide will stain only cells that have lost membrane integrity. Live cells will appear uniformly green. Early apoptotic cells will stain green and contain bright green dots in the nuclei as a consequence of chromatin condensation and nuclear fragmentation. Late apoptotic cells will also incorporate ethidium bromide and therefore stain orange but, in contrast to necrotic cells, the late apoptotic cells will show condensed and often fragmented nuclei. Necrotic cells stain orange, but have a nuclear morphology resembling that of viable cells, with no condensed chromatin. Histology of liver and kidney were seen.

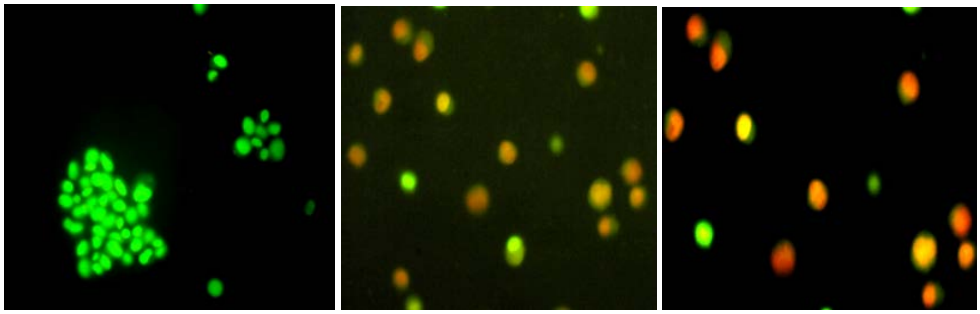
Statistical Analysis

All the experiments were repeated thrice. All data were expressed as mean ± S.D. The statistical significance was evaluated by one- way analysis of variance (ANOVA) using SPSS version 17 and the individual comparison were obtained by Duncan's Multiple range test (DMRT). A value of $P<0.05$ was considered to indicate a significant difference between groups. Values with identical letter are not significantly different according to Duncan's multiple range test (DMRT) at 5% level.

Results and Discussion

Ex vivo studies

Cell viability assessment (Tryphan blue staining) Cell viability assessment was carried out through the tryphan blue staining. Tumor cells were collected from the Dalton's lymphoma ascites bearing mice. *D. oppositifolia in vivo* and *in vitro* tuber extract and its nanoparticles, diosgenin, bioactive compound and its nanoparticles induced cell death in Dalton's lymphoma ascites tumor. Among the six, Diosgenin nanoparticles treatment induced significant cell death whereas, Diosgenin and tuber extract treated mice show less cytotoxic effect on cancer cells. (Fig. 1).



Control *In vitro* tuber *In vitro* tuber AgNps

Fig 1: Apoptotic morphological changes identified in cancer cells by Acridineorange/Ethidium bromide (AO/EB) staining. Greenish-yellow colour indicates early apoptotic cells and orange-red indicates late apoptotic cells. Here blebbing represents apoptotic cell death which had bright orange chromatin with round nuclei. Diosgenin SNPS treatment showed late apoptotic cells.

In vitro cytotoxicity assay - MTT Assay

In MTT Assay, *in vitro* cytotoxicity was performed against human Breast cancer cell line (MCF-7) using *in vivo* tuber and its nanoparticles, *in vitro* tuber and its nanoparticles, diosgenin and its nanoparticles. Among the test agents. *In vivo* tuber nanoparticles also have significant cytotoxic effect (99.5%) with IC_{50} value of 1.1 µl/ml whereas *in vivo* tuber does not show cytotoxicity (Table 26). *In vitro* tuber nanoparticles also have (88.8%) significant cytotoxic effect with IC_{50} value of 2.2 µl/ml whereas i*n vivo* tuber does not shown cytotoxicity.

In the present investigation, the potent cytotoxic activity of Diosgenin and their silver nanoparticles on MCF-7 cell line was observed by MTT assay. The IC 50 value of Diosgenin SNPS was 1.25µl/mg. The percentage of cell inhibition is higher in Silver nanoparticles than the Diosgenin. The Diosgenin mediated SNPS showed 100 % of cell inhibition was observed at 1.25µl/mg. The anticancer activity of the pure constituent isolated from *Stephania venosa* tuber on human ovarian cancer cells (SKOV3) (Montririttigri *et al*., 2008). Diosgenin, which is extremely very useful in future to solve many problems related to control cancer. This is the first report to synthesise the silver nanoparticles from Diosgenin with their cytotoxic effect and antitumor effect against Breast cancer cell line MCF-7 was evaluated. In histopathological studies, *Dioscorea oppositifolia in vivo* and *in vitro* tuber, Diosgenin and their silver nanoparticles treated animals had significant effect compared with Dalton's lymphoma ascites bearing mice. Diosgenin and their SNPS had very high effect than the tuber extract and their nanoparticles. Upon treatment with Diosgenin and their SNPS significant effect on proximal convoluted tubule, glomerulus, artery and vein were observable. *Dioscorea oppositifolia* L., *In vivo* as well as *in vitro* tuber extract and their nanoparticles treated mice had moderate effect on liver and kidney damage (Table 7&8).

Table 1: Cytotoxicity Effect of *In vitro* tuber and its SNPS against Human Breast cancer Cell line (MCF-7)

***In vitro* tuber**		***In vitro* tuber SNPS IC 50-2.24 µl/ml**	
Conc (µl/ml)	**% Cell Inhibition**	**Conc (µl/ml)**	**% Cell Inhibition**
18.75	2.521008	0.31	1.042753
37.5	11.11111	0.625	3.232534
75	13.81886	1.25	15.43274
150	15.12605	2.5	58.60271
300	17.27358	5	88.84254

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