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# Development of anti-cancer nano-formulations of Ocimum sps.

KEYWORDS

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**ABSTRACT** *For a drug to reach in the optimum amount to the infected region at "minimum effective level," it is necessary to investigate its therapeutic effect. A better understanding of the pharmacokinetics and bioavailability of phyto-pharmaceuticals may help in designing rational dosage regimens. This can be achieved by designing novel drug delivery system (NDDS) for herbal constituents. Nano-sized drug delivery system has potential applications for enhancing activity. Incorporation of herbal extracts into novel formulation system helps to increase bioavailability and stability, reduce toxicity and repeated administration. In this paper an attempt was made to develop nano-sized drug formulation of Ocuimum sanctum Linn, for its anti-cancer activity.*

## 1. Introduction

Herbal medicines are the basis of health care and have become a global importance from last decade as they play central role in health care system of large population [S. Rajeshwari, 1992]. In recent years, the nano-formulations of herbal medicines have attracted much attention [Sanyal PK, 1989]. Applications of nanotechnology for treatment, diagnosis, monitoring and control of biological system have recently referred to as nanomedicine [Pandey BP, 1990]. Though herbal medicines have many constituents that work against the diseases, they also show several adverse effects. Hence phyto-therapeutics needs a scientific approach to deliver the components in a sustained manner to increase the therapeutic activity [Nagarjun S, 1989, Reghunandana R, 1995]. The nano-particles have come forward as the capable approach in drug delivery systems for the well-organized delivery of drugs utilized in the treatment of various diseases such as cancer by crossing the reticulo endothelial system, enhanced permeability and retention effect, and tumor-specific targeting. Among the plants known for medicinal value, the plants of genus *Ocimum,* belonging to family *Labiatae* are very important for their therapeutic potentials [Vohra SB, 1969, Khanna S, 1981]. *Ocimum sanctum L.* (OS) more recently known as *Ocimum tenuiflorum* (= Tulsi in *'Sanskrit'*). The leaves of OS contain 0.7% volatile oil comprising about 71% eugenol and 20% methylated eugenol. Fresh leaves and stem of *OS* extract yielded some phenolic compounds (antioxidants) such as cirsilineol, circimaritin, isothymusin, apigenin and rosameric acid, and appreciable quantities of eugenol. [Sarkar A, 1990, Mandal S, 1993]. The anticancer activity of *OS* has been proved and cited by several investigators [Liv J, 1995]. The alcoholic extract of leaves of *OS* has a modulatory influence on carcinogen metabolizing enzymes such as cytochrome P 450, cytochrome b5, aryl hydrocarbon hydroxylase and glutathione Stransferase (GST), which are important in detoxification of carcinogens and mutagens [Nishijima H, 1999]. In this paper the nano-formulations of *Ocimum tenuiflorum* were evaluated for its *in vitro* anticancer activity against MCF-7 (Breast cancer cell line, malignant).

## 2. Materials and Methods

### 2.1 Collection of plant material

Fresh leaves (purple) of *Ocimum tenuiflorum* (Krishna Tulsi) were collected from surrounding areas of Visakhapatnam, India. The leaves were thoroughly washed in double distilled water for the removal of dust particles, shade dried and then ground to powder using mortar and pestle. The powdered samples were stored in airtight closed bottles for further experiments.

### 2.2 Preparation of plant extract of *ocimum tenuiflorum*

20gms of the plant powder of *Ocimum tenuiflorum* was taken in 250 ml, Erlenmeyer flask and boiled along with 100 ml of double distilled water. Further, the extract was filtered with Whatman Filter paper no. 1 after cooling and stored at 4°C for further experiments.

### 2.3 Synthesis of AgNPs

For the preparation of silver nanoparticles, 1mM AgNO3 (Sigma-Aldrich, India) solution was prepared.10 ml of the leaf extract obtained was added to 90 ml of silver nitrate solution under stirring. The solution was allowed to react at room temperature. Reduction of silver ions can be monitored by the color change due to surface plasma resonance. The aqueous silver nitrate solution was turned to pale yellow and then to dark brown color after the addition of *Ocimum sanctum* extract due to reduction of silver. Periodic sampling was carried after the color change to monitor the formation of AgNPs and allowed the reaction at room temperature for 24 hrs.

### 2.4 Characterization of AgNPs

#### 2.4.1 UV-Visible spectral analysis

Synthesized silver nano-particles were analyzed to determine maximum production of silver nano-particles by sampling the aliquots withdrawn from reaction mixture at

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different time intervals. The absorption spectra of the samples were taken after the intensification of color. The absorption spectra were carried by UV-Vis spectrophotometer, (Eppendroff Biospectrophotometer, India) as a function of reaction time at room temperature at the wavelength of 400 – 800 nm. Path length of 10mm cuvette was used where de-ionized water used as blank. The readings were noted from 15min -90min.

**2.4.2 FT-IR analysis**

The bio reduced silver nitrate solution was centrifuged at 10,000 rpm for 15 min. The centrifugation was carried out for 2-3 times with distilled water for purification of the silver nanoparticles. The supernatant was discarded and the pellet was dried. The dried samples were grinded with KBr pellets and used for FTIR (Perkin Elmer Spectrum 2) measurements to know the binding properties. The spectrum was recorded in the range of 4000 - 400 cm-1.

**2.4.3 XRD analysis**

To monitor the formation and quality of compounds obtained AgNPs were checked by X-ray diffraction (Panalytical, X-Pert Pro) spectrum. The XRD pattern was measured by drop coated films of AgNO3 on glass plate and employed with X-ray diffractometer in the range of 20° to 70°.

**2.5 Anticancer Activity of *O.tenuiflorum* against MCF-7 cell line**

**MTT Assay**

To investigate the invitro inhibitory effects of the AgNPs synthesized from leaf extract of *O.tenuiflorum*, MCF-7(Breast cancer, malignant) was procured from NCCS, Pune, India and sensitivity of MCF-7 to nano-formulated *Ocimum tenuiflorum* was determined by the MTT colorimetric assay. 5000 10000 cells approximately in 100 µl MEM media (MEM199, Sigma, India) per well was seeded in a 96 well plate and incubated at 37°c, 5% CO2. The cells were exposed to nanoformulted and leaf extract of ocimum tenuiflorum at different concentrations from 6.25µg/ml to 200µg/ml. Standard drug Temoxiflon and the solvent DMSO treated cells served as control. The drug was allowed to take effect by incubating at 37°c, 5%CO2. The cells were then treated with, 20µl of freshly prepared MTT reagent (5mg/ml in PBS) was added and then DMSO (200 µl) was added to each well to dissolve the formazan crystals. The absorbance (OD) of the culture plate was read at a wavelength of 492 nm on an ELISA reader, Anthos Biochrom 2020 ELISA Reader. Percentage of residual cell viability was determined.

**2.6. DNA Laddering on *O.tenuiflorum* treated Lymphocytes**:

DNA Laddering was performed to identify the toxicity levels of the synthesized AgNPs by *Ocimum tenuiflorum* on normal cells. Fresh healthy blood sample was collected and the lymphocytes were treated with aqueous extract and nano-formulated ocimum sanctum with four different concentrations of 25, 50, 100, 200 µg/ml and incubated at 37°C for 48hr. DNA was isolated by simple, rapid, non-enzymatic method (Lahiri and Nurnberger, 1991). Gel Electrophoresis was performed using 1% agarose gel (1.0 gm of agarose in 100ml TEB 1X buffer) the gel was observed immediately in the UV transilluminator for DNA bands.

**3. Results and Discussions**

**3.1. Synthesis of AgNPs**

Formation of AgNPs can be easily monitored from change in colour of reaction mixture during exposure to *Ocimum tenuiflorum* leaf extract. Change in colour presented in (Figure.1) indicates the formation of AgNPs. A characteristic colour change was observed upon addition of *ocimum tenuiflorum* leaf extract to 1mM silver nitrate solution which resulted from dark yellowish and to brownish black, due to excitation of surface plasma resonance. Presence of water soluble reducing agents in *Ocimum sanctum* leaf extract was the reason for formation of silver nanoparticles. Reduction of silver ions to silver particles after exposure plant extract followed by colour change to dark brown suggests formation of AgNPs.

**Figure.1 Aqueous solution of AgNO3 before and after addition of leaf extract of *Ocimum sanctum* .**

**3.2 Characterization of AgNPs**

**3.2.1 UV-Visible Spectral Analysis**

In order to monitor the formation of silver nano-particles UV-Visible absorption spectra was recorded as a function of reaction time. The UV results shows silver nanoparticle exhibited maximum absorption peak at 429nm (Figure.2). From the earlier reports it was observed that absorbance at around 430nm is characteristic feature of metal particles (Nestor et al., 2008). The free electrons present in metal nano-particles gives rise to Surface Plasmon Resonance (SPR) absorption band. The flat curve shows the polydispersion of nano-particles in solution. From the study it is evident that reduction of silver ions was quite rapid due to the presence of potent antioxidants activity of *Ocimum tenuiflorum* extract.

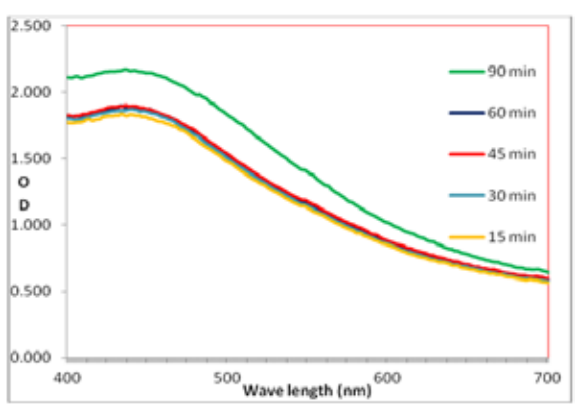


**Figure.2 UV-Vis spectra of AgNPs synthesized by ocimum sanctum**

**3.2.2. FT-IRspectroscopy**

FTIR measurements were carried out to identify the biomolecules for capping and efficient stabilization of the metal nano-particles synthesized by *Ocimum tenuiflorum* leaf broth. The FTIR spectrum of silver nano-particles is shown in Fig. 5. The band at 3435 cm-1 corresponds to O-H stretch H-bonded alcohols and phenols. The peak at 2921 and 2851 cm-1 corresponds to O-H stretch carboxylic acids. The assignment at 1620 cm-1 corresponds to C -0 stretches. The peak at 1321 cm-1 corresponds to C-N

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stretching of aromatic amine group and the bands observed at 1101, 1019, cm-1 corresponds to C-N stretching alcohols, carboxylic acids, ethers and esters. Therefore the synthesized nano-particles were surrounded by proteins and metabolites such as terpenoids having functional groups of alcohols, ketons, aldehydes and carboxylic acids. From the analysis of FTIR studies the carbonyl group from the amino acid residues and proteins has the stronger ability to bind metal indicating that the proteins could possibly from the metal nano-particles (i.e., capping of silver nanoparticles)

**Figure.3 FTIR Spectrum of AgNPs Synthesized by Tulsi extract.**

**3.2.3 XRD analysis**

The XRD spectrum in Figure.4 showed six distinct diffraction peaks. The obtained data was analysed using Joint Committee on Powder Diffraction Standards. Peak 1 of figure 4 represents Silver Nitrate Urea ($AgNO_3(NH_2)_2CO$) or Silver Picolinate Hydrate ($C_{12}H_9AgN_2O_4.H_2O$) where as Silver Imide Carbonate ($Ag_2NHCO_2$) and Silver Nicotinate ($AgC_{12}H_8N_2O_4$) corresponds to Peak 2 and Peak 3 respectively. The assignment of Peak 4 and peak 5 represents about $Ag_2O_3$ and Silver Nitride ($AgN_3$). Peak 6 corresponds to Silver Ketanide ($C_2Ag_2O$).

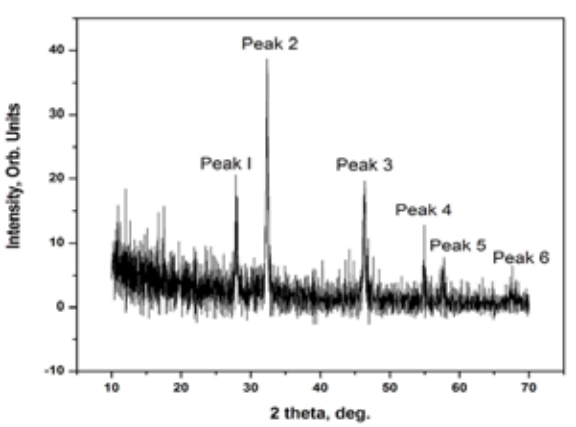


**Figure 4.XRD Pattern**

**3.4 Anti cancer activity of *O.tenuiflorum* against MCF-7 cell line**

**MTT assay**

The anticancer activity of silver nano-particles was investigated by MTT assay. From the Table.1 it can be observed that AgNPs from aqueous extract of *ocimum tenuiflorum* when subjected to different concentrations showed highest cell inhibition of 82.69% at 200µg/ml. While aqueous extract showed cell inhibition of 62.43% at 200µg/ml. These were compared with standard drug Tamoxifen which was shown in Table 2. Figure.5 shows the graphical representation of aqueous extract and AgNPs synthesized by the *ocimum tenuiflorum* along with the standard.

Blank=0.040 control=0.553

| Concentration (mg/ml) | Aqueous extract of *ocimum sanctum on* ( MCF-7) | | | AgNPs synthesized *by ocimum sanctum* (MCF-7) | | |
|---|---|---|---|---|---|---|
| | O.D at 492nm | % of cell survival | % of cells under apoptosis | O.D at 492nm | % of cell survival | % of cells under apoptosis |
| 6.25 | 0.551 | 92.04 | 7.96 | 0.509 | 84.81 | 15.19 |
| 12.5 | 0.484 | 80.28 | 19.71 | 0.504 | 83.90 | 16.10 |
| 25 | 0.474 | 78.48 | 21.51 | 0.431 | 70.70 | 29.25 |
| 50 | 0.465 | 76.85 | 23.14 | 0.384 | 62.20 | 37.79 |
| 100 | 0.328 | 52.08 | 47.92 | 0.260 | 39.85 | 60.14 |
| 200 | 0.198 | 28.57 | 62.43 | 0.135 | 17.30 | 82.69 |

**Table2:Effect of aqueous and AgNPs synthesized by *ocimum tenuiflorum* on MCF-7 cell line**

| Concentration (µg/ml) | Tamoxifen ( standard drug) | | |
|---|---|---|---|
| | O.D at 492nm | % of cell survival | % of cell under apoptosis |
| 6.25 | 0.401 | 88.5 | 11.5 |
| 12.5 | 0.348 | 75.5 | 24.5 |
| 25 | 0.215 | 42.9 | 57.1 |
| 50 | 0.153 | 27.7 | 72.3 |
| 100 | 0.024 | 3.90 | 96.1 |
| 200 | 0.007 | 0.08 | 99.92 |

**Table 3: Effect of Tamoxifen on MCF-7 cell line. Blank: 0.040 Control = 0.448**

**Effect of aqueous extract and nanomulation of Ocimum tenuiflorum on MCF-7 cell line**

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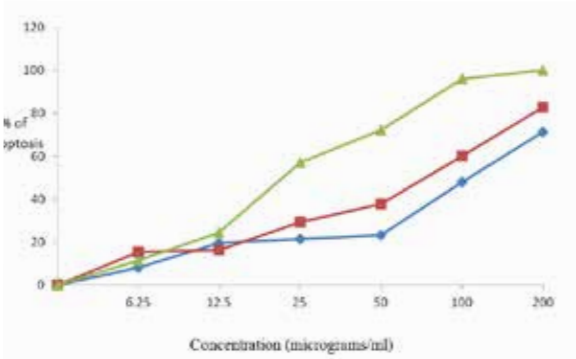


**Figure.5 : Effect of aqueous extract and AgNPs synthesized by *Ocimum.tenuiflorum* in comparison with standard Temoxifen anticancer activity against MCF-7 cell line.**

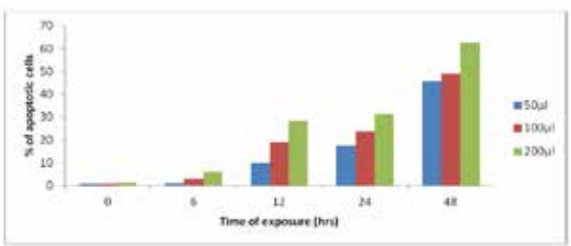


**Figure 6: % of apoptotic cells when treated with leaf extract of *Ocimum tenuiflorum***

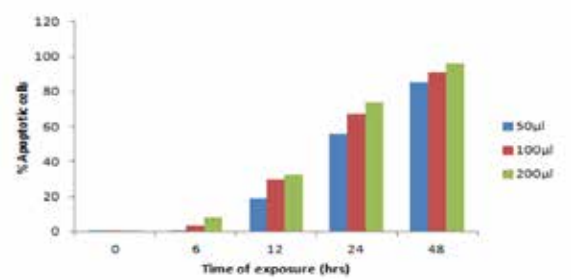


**Figure 7. % of apoptotic cells when treated with nano-formulated *Ocimum tenuiflorum* on MCF-7(Breast cancer cells)**

### 3.5. DNA Laddering

The effect of the synthesized AgNPs and aqueous extract by *ocimum sanctum* on normal cells was shown in the (figure.9). The test was conducted for the assessment of toxicity levels at the biochemical level for apoptosis. Submarine gel electrophoresis provides a rapid and convenient way to check the quality of DNA and its physical state. Electrophoresis analysis of DNA using agarose gel can confirm DNA integrity. Smears on gel indicate fragmented DNA and intact DNA will give a clear band. It shows there is no toxic effect on normal cells as there is no DNA fragmentation.

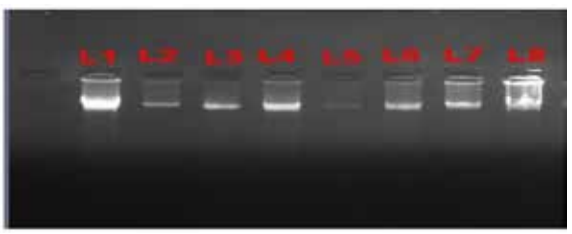


**Figure 8 Mononuclear cells when treated with aqueous extract and AgNPs synthesized by** *ocimum tenuiflorum*

Lane 1-4: Aqueous extract of *Ocimum tenuiflorum*

Lane 5-8: AgNPs synthesized from aqueous extract of *ocimum teuniflorum*

### 4. Conclusion:

Very few reports have been stated about the anticancer activity of *Ocimum tenuiflorum* against breast cancer cells. Taking the cited facts into consideration the present study showed that a nano formulation of silver particles of Ocimum sanctum had considerable anti –cancer activity against breast cancer cell lines. These results have shown us a path to conduct in vivo experiments to evaluate the nano-formulations of Indian Tulsi.

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