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RESEARCH ARTICLE

IN VITRO ANTICANCER AND ANTIPLATELET ACTIVITY OF ZINC OXIDE NANOPARTICLE SYNTHESISED SEED EXTRACTS OF *CELOSIA ARGENTEA* L.

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ABSTRACT

The objective of the present study was to synthesis of nanoparticles in plant extract which gained considerable importance when compared to bulk counterparts. Among many nanoparticles, zinc oxide (ZnO) nanoparticles are very much important due to their utilization in drug-delivery system. In present study *Celosia argentea* L seeds were selected to synthesise zinc oxide nanoparticles and to evaluate its anticancer and antiplatelet activity. From the results it can be concluded that *Celosia argentea* L seeds extract exhibited significant antioxidant activity and it might be due to various phytoconstituents in it.

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INTRODUCTION

Nature is the source of medicinal agent for thousands years and an impressive number of modern drug have been isolated from natural sources, many of these medicines. Medicinal herb is considered to be a chemical factory as it contains multitude of chemical compounds like alkaloids, glycosides, saponins, resins. A substantial number of drugs are developed from plants. A large proportion of such drugs have been discovered with the aid of ethno botanical knowledge of the traditional uses of the plant. The World Health Organization (WHO) estimates that 80% of the world populations presently use herbal medicine for primary health care (Preethi *et al.,* 2015). Nanotechnology is making an impact in every field of life. Researchers are expanding their interest towards synthesis of nanoparticles. For past few decades, metal nanoparticles have elicited much interest due to their distinct physical, chemical and biological properties and had become most active area of research (Shakeel Ahmed *et al.*, 2015). Zinc oxide nanoparticles have gained immense popularity in the scientific world due to their distinctive and fascinating properties. The synthesis and assembly of nanoparticles would benefit from the development of clean, nontoxic and environmentally acceptable "green chemistry" approaches for nanoparticles (Ramesh *et al*., 2014).

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Highly reactive free radicals and oxygen species can initiate degenerative diseases. They arise from metabolism or environmental sources interact continuously in biological systems and uncontrolled generation correlates directly with molecular level of many diseases (Urmila *et al*., 2013). Antioxidants are vital substances, which possess the ability to protect the body from damages caused by free radical induced oxidative stress.

The antioxidant at low concentrations significantly delays or prevents oxidation of substrate. Biological antioxidants have been defined as compounds that protect biological systems against the potentially harmful effects of processes or reaction that can cause excessive oxidation (Urmila *et al*., 2013). *Celosia argentea* Linn belongs to family Amaranthaceae. In India, it is found to be grown as a weed of bajara fields. There are 17 species of celosia recorded in India. Seeds traditionally used for treatment of jaundice, gonorrhea, wounds and fever. Whole plant is used as antidote for snake-poison. Flowers and seeds are used for bloody stool, hemorrhoidal, bleeding, leucorrhea and diarrhea. Root used for colic, gonorrhea and eczema (Thakur Hemantkumar, 2016). Seeds are used to relieve gastrointestinal disorders and are antipyretic. The juice of the seeds forced into the nostrils is a cure for epistaxis. Root used for colic, gonorrhea and eczema (Naeemullah Khan, 2016).

Figure 1. ***Celosia argentea*** **flower and seeds**

MATERIALS AND METHODS

Collection and preparation of plant extract

The plant *Celosia argentea* was collected from different localities of Coimbatore district. It was identified and authenticated by Botanical Survey of India, (BSI/SRC/5/23/2016/Tech/2115), Tamilnadu Agricultural University, Coimbatore, Tamilnadu, India. The seeds were freshly collected and 3g of it was crushed in mortar and pestle to fine powder. Six different solvents were used in the current study such as aqueous, ethanol, hydro ethanol, chloroform, acetone, petroleum ether. To the fine powder 50ml of each solvent were added and kept in a shaker for 24hrs. Then they were filtered separately by using muslin cloth. The filtrates were stored in refrigerator at 4˚C for further use.

Synthesis of ZnO nanoparticles

100ml of 100mM Zinc nitrate solution is prepared and kept on a magnetic stirrer set at 60˚C and 750 rpm.15 ml of leaf extract is added in a dropwise manner and the color change is observed. pH is checked and adjusted to 12 by the addition of a 1M solution of NaOH. Observation of white cloudy appearance marks the formation of ZnO nanoparticles formation. The solution is left for two hours in same condition. Incubate overnight at room temperature. Centrifuge at 5000 rpm for 20 minutes, the White pellet is collected and dried in an oven at 150˚C. White dried powder is obtained, which was collected for further use. The filtrates were stored in refrigerator at 4˚C for further use.

In vitro evaluation of anticancer activity of *Celosia argentea* seeds

Cell line

The human Breast cancer cell line (MCF7) 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% CO_2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

EVALUATION OF CYTOTOXICITY

MTT assay

3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. After 48 h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The percentage cell viability was then calculated with respect to control as follows % Cell viability = [A] Test / [A]control x 100. The % cell inhibition was determined using the following formula.

% Cell Inhibition = 100- Abs (sample)/Abs (control) x100

Nonlinear regression graph was plotted between % Cell inhibition and Log concentration and IC_{50} was determined using Graph Pad Prism software.

Antiplatelet activity

Platelet-rich plasma and Tyrode buffer were used for the antiplatelet activity according to Iman *et al.* (2009). Platelet-rich plasma (PRP) was prepared by centrifugation of citrated blood at 22°C for 6min, at 400g. Platelets were adjusted to 3.0×108 cell/ml with sterile saline. Tyrode buffer was prepared using sodium chloride 149mM, potassium chloride 2.6mM, sodium bicarbonate 9.5mM, glucose 5.5mM, sodium dihydrogen phosphate 0.5mM, magnesium chloride 0.6mM and gelatin 0.25%. The platelet-rich plasma 0.13 × 10-7 for each assay was resuspended in Tyrode buffer (pH adjusted to 7.4 with 0.25 M Hcl). Aggregation of the platelets was induced by Antiplatelet activity of plant extracts with $CaCl_2$ at a final concentration of 2µM. Platelet aggregation recorded by increasing transmittance value of spectrophotometric measurements. To determine the *in vitro* antiplatelet aggregation property, different concentrations (100, 200, 300, 400 and 500µg/ml) of plant extract were added to the platelet suspension for 1min exposure at 37ºC before treatment with platelet aggregating agents. Aspirin at 500µg/ml was used as a standard.

RESULTS

The formation of ZnONPs was confirmed with appearance of turbid yellow solution and change in Surface Plasmon Resonance (SPR) UV–vis spectroscopic analysis. The UV–vis spectra was recorded at 300 nm for hydroethanolic extract and 400nm for aqueous extract corresponds for the formation of ZnONP's.

In vitro evaluation of anticancer activity of *Celosia argentea* seeds

The effect of synthesized nanoparticles on MCF-7 cancer cell line is shown in figure-2. *In vitro* cytotoxic activity of *Celosia argentea* seed extract at 18.5, 37.5,75 µg concentrations showed a potent activity against selected cancer cell line. These observations may be due to the presence of active biological compounds. The results clearly demonstrate that the all the compound has the ability to inhibit cell proliferation in a dose dependent manner. The IC_{50} values of compounds against human breast cancer cells were found to be 174.29µM/ml. The exposure of MCF7 cells to ZnO nanoparticles synthesized *Celosia argentea* at the various concentrations significantly reduced the cell viability in a concentration dependent manner.

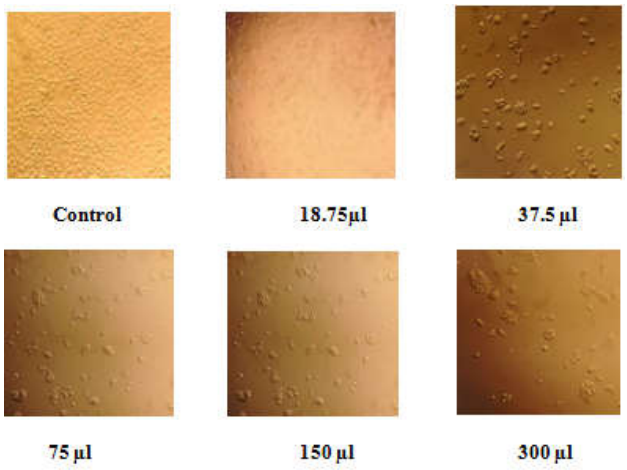

Figure 2. Cytotoxicity assay (MTT)

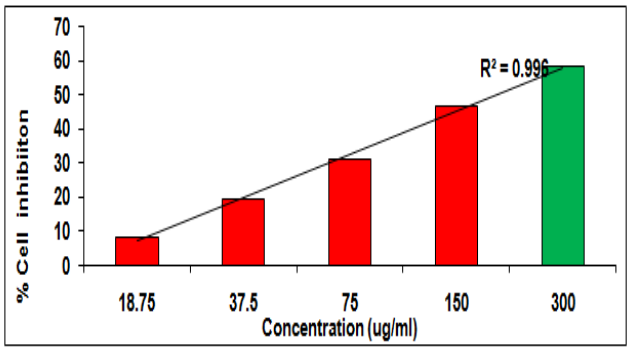

Figure 3. Percentage of cell inhibition against MCF7 cell line in ZnO nanoparticles

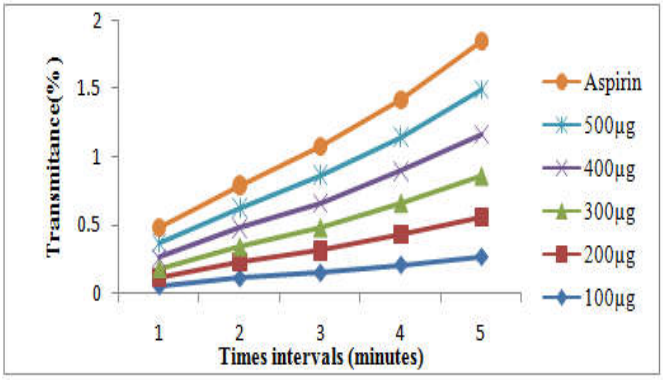

Figure 4. Antiplatelet activity of hydroethanolic extract of *Celosia argentea* seeds

However the cell viability at the higher concentration (1000 µg/ml) was not found to be significant. As the concentration increases the percentage cell inhibition was found to be significantly increased. Rukhsana *et al*.,2015 reported that the *Celosia argentea* exerted potent anticancer activity against MCF7 with inhibitory concentration of 28 µg/ml ($P<0.001$) with no toxicity towards the normal cells.

EVALUTION OF ANTIPLATELET

Activation of platelets plays a key role in haemostasis and circulation. Platelet dysfunction contributes to the development and progression of cardiovascular diseases such as arterial hypertension, altherosclerosis and thrombosis.

Indeed, it has been reported that patients with hypertension or coronary heart disease tend to have increased platelet reactivity.

DISCUSSION

The medicinal properties of plant could be attributed to the presence of bioactive compounds. Phytochemical analysis of *Celosia argentea* seeds carried out in various solvents extracts shown remarkable phytochemical constituents. The experimental results demonstrate that the all the compound has the ability to inhibited cell proliferation in a dose dependent manner. . As the concentration increases the cell inhibition % significantly increases. *Celosia argentea* seeds therefore possess potent anticancer activity against against human breast cancer cells (MCF 7). The results were due to the presence of phytochemical constituents in it. Many investigations were carried out towards the prevention of the abnormal hyperactivity of platelets from the results of antiplatelet assay it is evident that the hydroethanolic seed extract of *Celosia argentea* L showed effective antiplatelet activity in a dose-dependent manner with maximum activity at 500µg/ml concentration.

Conclusion

The results of this study support the efficacy of *Celosia argentea* seeds as an anticancer agent against MCF-7 Breast cancer cell lines. The anticancer activity was found due to the presence of phytochemical constituents and in combination with ZnO nanoparticle. Antiplatelet activity of *Celosia argentea* found to possess potent antiplatelet activity which might be due to the presence of phytochemical constituents in it. Further works on the *in vivo* anti-cancer activities of *Celosia argetea* seed extract needs to be assessed.

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