Antiproliferative efficacy of sorghum bran extracts against MCF-7 breast cancer cell lines

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Abstract
Over the past decade, there has been an increasing interest in using nanotechnology for cancer therapy. The development of smart targeted nanoparticles (NPs) that can deliver drugs at a sustained rate directly to cancer cells may provide better efficacy and lower toxicity for treating primary and advanced metastatic tumors. The present study was aimed to investigate the in vitro cytotoxicity effect of biogenic Zinc nanoparticles ZnONPs against human breast cancer (MCF-7) cells towards the development of anticancer agent. Nanobiocomposite of zinc oxide nanoparticles conjugated with Sorghum Bran was produced and was confirmed using maximum UV–Vis absorption at 340 nm in the present work. The anti-cancerous nature of the synthesized zinc oxide conjugated nanobiocomposite on MCF-7 cell line was studied using MTT assay. The viability of the MCF-7 cells was decreased to 48% when it was treated with Sorghum bran conjugated zinc oxide nanobiocomposite. There was an immediate induction of cellular damage in terms of loss of cell membrane integrity, oxidative stress and apoptosis were found in the cell which treated with ZnONPs. This may be a first report on anti-MCF-7 property of biogenic ZnONPs, but mechanism needs to be elucidated.

Keywords: Sorghum, MTT assay, anticancer

1. Introduction
Medicinal plants and its herbal therapy have their important role in the treatment of various diseases. Besides their therapeutic potency, some of the medicinal plant has toxicity effect particularly in children and elderly people. So it is believed that medicinal plants are the natural products which are safe to consume and also less toxic. Traditional medicines promote the health maintenance and diagnose the diseases. The current research behind the medicinal plant therapy includes the mind, body and energy based healing, herbal remedies [1]. Ethanopharmacological screening of medicinal plants is considered to be an important approach where several medicinal plants have been reported as nontropic that alters the mental cognitive functions by influencing various different pathway of brain [2]. In latest advancement, the medicinal plants are replaced by modern drugs. Many people used to consume the scientifically developed tablets and pills. Medicinal plant usage creates the recent awareness among the people to treat diseases and drug that is developed from bark, seed, fruit and other parts of body. It also acknowledged that their active action and it has been included in modern pharmacotherapy [3]. Secondary metabolites of medicinal plants play an important role in diagnosis of disease and this in turn is used for production of novel medicines. Large number of plants possesses many other activities such as antioxidant, anti-inflammatory, antibiotic, anti insecticidal. Those are widely used by the people all around the world. Evaluating the active plant derived compound, herbal drugs can help in the healthcare system to treat human diseases in future [4]. Sorghum is a world’s fifth largest produced cereal after maize, rice, wheat and barley. Sorghum is also rich in bioactive compounds particularly 3-deoxyanthocyanins, tannins, phenolic acids and minerals such as calcium, phosphorous, iron, zinc and magnesium ,unsaturated lipids and complex B vitamins. Sorghum bran, that is usually discarded s waste, is identified to be rich in phenolic compounds.contains a phenolic compounds in the form of phenolic acids, flavonoids and condensed tannins which have antioxidant activity [5]. Anthocyanins are the poly phenolic pigments that belongs to a flavonoid group and it is responsible for the red-orange to blue-violet colours present in plant organs. They play relevant roles in plant propagation and ecophysiology and plant defense mechanisms and are responsible for the colour of fruits and vegetables. A large number of novel anthocyanin structures have been identified, including new families such as pyranoanthocyanins or anthocyanin oligomers; their biosynthesis pathways have been elucidated [6]. Furthermore, evidence about their benefits in human health has accumulated, and processes of anthocyanin absorption and biotransformation in the human organism have
started to be ascertained. Earlier, anthocyanin is only known for the colouring properties and now anthocyanin plays an important role in the health benefits as dietary antioxidants by preventing neuronal diseases, cancer, diabetes, cardiovascular illness, inflammation and many other diseases [7]. The present study focuses on the isolation of anthocyanins from Sorghum bran, convert to a ZnO nanoparticle and evaluate its efficacy as anticancer in in vitro models.

2. Methodology

2.1 Preparation of Extract of the Sample

The Sorghum plant was collected from farming land in Coimbatore. The grains in the plants are removed and the bran is separated from the plant. The obtained bran was powdered. 10g of bran powder were taken in a conical flask for sample extraction by using 100ml of chloroform solvent for 72hrs. After the incubation, the extract was air dried. The crude that was obtained was dissolved in water and used for studies.

2.2 Synthesis of Zinc Oxide Nanoparticles

In a typical reaction mixture, 5-10 ml of the aqueous extract of red sorghum bran was added to 300 ml of 4Mm of aqueous zinc sulphate heptahydrate solution and stirred at room temperature for 5 min s to achieve the pale yellow solution. After that, 1M sodium hydroxide solution is added to the mixture drop by drop with continuous stirring at room temperature. The yellow colour of the above mixture starts change to yellowish suspension at pH 12. The suspended particles were purified by dispensing in sterile distilled water and centrifuged thrice. Further, the white precipitate particles were washed with ethanol to remove the impurities for the final product. The white powder was obtained after drying at 60°C in vaccum oven for 6 hrs. The formed nanocrystals were confirmed by its UV Vis absorption at 340nms.

2.3 In vitro screening of Red Sorghum branZnOPs on MCF-7 Cancer cell line by MTT Assay [8]

2.3.1 Cell line and culture

MCF 7 cell line was obtained from National Centre for Cell Sciences, Pune (NCCS). The cells were maintained in DMEM supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO2 at 37°C.

2.3.2 Procedure

Cells (1 × 10⁵/well) were plated in 24-well plates and incubated in 37°C with 5% CO2 condition. After the cell reaches the confluence, the various concentrations of the samples were added and incubated for 4hrs. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or DMEM without serum. 100µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazoly)-2,5-diphenyl–tetrazolium bromide (MTT) was added and incubated for 4 hours. After incubation, 1ml of DMSO was added in all the wells. The absorbance at 570nm was measured with UV-Spectrophotometer using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition (IC50) was determined graphically. The % cell viability was calculated using the following formula:

\[
\text{% Cell viability} = \frac{A570 \text{ of treated cells}}{A570 \text{ of control cells}} \times 100 \\
\]

Graphs are plotted using the % of Cell Viability at Y-axis and concentration of the sample in X-axis. Cell control and sample control is included in each assay to compare the full cell viability assessment.

3. Results and Discussion

Pharmacological studies fused with the many clinical observations and their effects of chronic administration of chemotherapy agent for the cancer the cancer treatment. Anticancer drugs cause an irreversible pharmacological response, where it requires long observation times and it is not measurable accurately. The empirical approach to cancer chemotherapy has often been successful [9]. Dietary antioxidants and various phytochemicals occurring in the herbs to prevent cancer that induces oxidative damages. Oxidative stress is a component of environmental toxicity during the cancer process. ROS potentially play an important role in the cancer. The level of ROS determines the risk of cancer in humans. Low level of ROS can be beneficial in the normal function, whereas there is the increased risk of cancer if occurs the chronic exposure to ROS. Increased level of ROS tends to induce apoptosis and cell death in various types of cancer. The antioxidant activity of phytochemicals is determined and used to prevent cancer [10]. Results obtained from the previous phases prove that, nanoparticles of red sorghum bran (ZnONPs) was found to exert better free radical scavenging, antioxidant effects in all the in vitro models.

3.1 MTT ASSAY

The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay is based on the conversion of MTT into formazan crystals by living cells, which determines mitochondrial activity. Since for most cell populations the total mitochondrial activity is related to the number of viable cells, this assay is broadly used to measure the in vitro cytotoxic effects of drugs on cell lines or primary patient cells. MTT assay allowing one to detect cell stress upon exposure to a toxic agent in the absence of direct cell death [11].

To determine the anticancer effect of ZnONP’s of red sorghum bran, MCF7 cell lines were used. Effect of synthesized nanoparticles on cell response of the Human breast cancer (MCF-7) cell line by using UV-vis spectrophotometer MTT assay. When the MCF7 cell lines were exposed to different concentration of ZnONP’s of red sorghum bran (7.8 µg/ml to 1000 µg/ml, the cell viability of the line decreased from 48.60% to 70.08%. The cell control have the absorbance of optical density at 0.395 and cell viability of 100%. IC50 value is found to be 7.8µg/ml [12].

![Normal cell line image](http://www.florajournal.com)
It is reported in previous study that Pp-ZnO NPs also effectively inhibited the biofilm formation of C. albicans at 50 µg ml$^{-1}$. Cytotoxicity studies revealed that a single treatment with Pp-ZnO NPs significantly reduced the cell viability of breast cancer cell line MCF-7 cells at doses higher than 50 µg ml$^{-1}$.[13, 14].

4. Conclusion
The in vitro antiproliferative and antiapoptotic activity of red sorghum bran ZnONPs were assessed against breast cancer cell line. The cytotoxic effect induced by the ZnONPs were also compared with control cells. The results obtained for viability and cytotoxicity assays showed that the exposure of cancer cells to red sorghum bran caused a steep decrease in the viability of the cells. The antiproliferative effect of red sorghum bran ZnONPs were found to be dose-dependent in the cell line. The present study highlights the efficacy of Sorghum bran as antiproliferative, but the exact molecular mechanism needs to be elucidated.

5. References

Table 1: Shows the anticancer effect of ZnONP’s of red sorghum bran on MCF7 cell line

<table>
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<tr>
<th>S. No</th>
<th>Concentration (µg/ml)</th>
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<th>Absorbance (O.D)</th>
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Fig 1: Represents the cell viability in %

Cell Viability (%)