

Research Article

Anticancer Activity of *Zingiber officinale* Bound Dendrimer - An *Invitro* Analysis

Indhumathy. M, Bavanilathamuthiah*, Carlin Geor Malar

Department of Biotechnology, Sathyabama University

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ABSTRACT

Zingiber officinale (sukku), one of the naturally occurring medicinal plants, has been consumed worldwide as a spice and flavoring agent from the ancient time. *Zingiber officinale* bears an enormous number of pharmacological activities including Neuro-protective activity and anticancer activity facilitating the extent of further research for finding out less toxic and more potent drugs for the better treatment of those diseases. Dendrimers are nanostructured macromolecules, immensely branched; three dimensional displays an essential role in the emerging field of Nanomedicine. Dendrimers have potential application in gene and drug delivery system. We examine the interaction of DNZ's (*zingiber officinale* bound Dendrimer) by UV – visible spectroscopy and Fourier transform infrared spectroscopy (FTIR). The anticancer activity of DNZ's was estimated against HaCaT and HEP 2 compared with Dendrimer and *zingiber officinale*. The IC₅₀ values of DNZ's were 63 µg/ml for normal cell line (HaCaT) and 32 µg/ml for cancer cell line (HEP 2). This shows that DNZ's has high anticancer activity compared to naked Dendrimers and *zingiber officinale*. The results indicate that functionalization of *zingiber officinale* with Dendrimer and successive solubilization could play an important role in the enhancement of anticancer activity.

Keywords: Dendrimer, *zingiber officinale*, anticancer activity, HaCaT, HEP 2.

INTRODUCTION

Polymeric drug delivery has intrinsically poor water solubility and high toxicity^{1,2}. Dendrimers are a class of highly branched polymers which act as an effective drug delivery vehicles due to their monodispersity and nanoscopic size³. A dendrimer can be synthesized with different functional groups in order to control the properties such as solubility, thermal stability etc. There are two methods of Dendrimer synthesis viz. divergent method and convergent method. The main properties of Dendrimer include high degree of uniformity, porosity, solubility and highly functional terminal groups. Their reactivity can also be rigorously controlled⁴. Dendrimer based nanotherapeutics can be employed mostly in the field of oncology as diagnosis and therapy. Dendrimer can be applied in several cancer therapies in order to improve their safety and efficacy⁵.

Cancer is one of the third leading diseases worldwide, which is preceded by cardiovascular and infectious diseases⁶. Ginger, (*Zingiber officinale* Roscoe, Zingiberaceae) is one of the naturally occurring important medicinal plant which is used as a spice and flavoring agent from the ancient time. It is an excellent source of several phenolics, including non-volatile compounds such as gingerols, gingerones etc⁷. The phenolics of sukku have been shown to display antioxidant⁸, anticancer⁹, anti-inflammatory¹⁰, anti-angiogenesis^{11, 12} and anti-atherosclerotic¹³ properties indicating its promising role as a chemopreventive agent.

In this present study, we aimed at evaluating the free *zingiber officinale*, Dendrimer and *zingiber officinale* bound Dendrimer effect on Anticancer activity against normal keratinocyte cells and laryngeal carcinoma cell lines.

MATERIALS AND METHODS

Chemicals

Oleoyl chloride was purchased from Sigma-Aldrich (Bangalore, India). Polyethylene glycol 400 (PEG 400), chloroform, acetone, methanol and dimethyl sulphoxide and triethylamine were the products from Merck (Mumbai, India). The cell culture medium (DMEM), fetal bovine serum (FBS) and the antibiotics were purchased from Himedia (Mumbai). All the chemicals used were of



Figure 1: Dried rhizome of *Zingiber officinale*

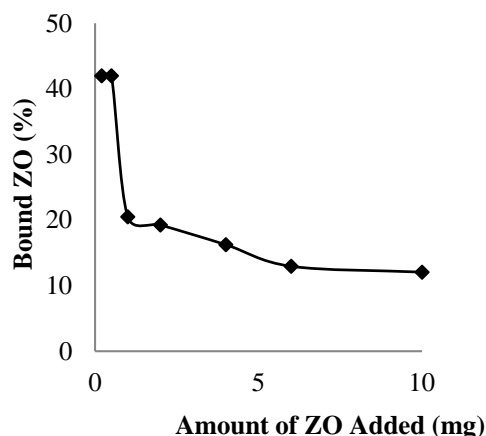


Figure 2: Binding efficiency of *Zingiber officinale* on dendrimer

analytical grade and Millipore water was used for all the experimental studies.

Plant material

The powders of Sukku (*Zingiber officinale* Roscoe, Zingiberaceae) were purchased from local market. The powdered material was extracted with acetone, dried under reduced pressure by using rotary evaporator. The residue was then stored in a desiccator. The dried rhizome of *Zingiber officinale* is as shown in fig.1.

Maintenance of cell lines

Normal cell lines such as HaCaT (normal keratinocyte cells) and human cancer cell lines such as HEp 2 (laryngeal carcinoma) was obtained from NCCS, Pune. The HaCaT were cultured in DMEM medium was supplemented with 10% heat inactivated fetal bovine serum and antibiotics. The HEp 2 cells were cultured in

DMEM medium was supplemented with 10% heat inactivated fetal bovine serum and antibiotics. The cell lines were maintained at 37 °C in a 5% CO₂ incubator and the media were changed frequently.

Synthesis of Dendrimer (D)

About 0.012 mol of triethylamine, 0.01 mol of polyethylene glycol 400 and 0.01 mol of oleoyl chloride was esterified in the presence of chloroform for 4 hours at 25 °C and the organic phase was removed. The collected Dendrimers were dried under vacuum until completely dried¹⁴.

Synthesis of zingiber officinale bound Dendrimer (DNZ's)

The amount of *zingiber officinale* was increased (2 to 10 mg) keeping the amount of the dendrimer as constant (50 mg). When 0.5mg of *zingiber officinale* was added to the 50mg of dendrimer, the bound *zingiber officinale* was found to be about 42%. There was high level of binding at lower loadings of *Zingiber*. Figure 2 shows the binding efficiency of varying *zingiber officinale* added to dendrimers. (ZO: *zingiber officinale*).

Characterization

The binding of zingiber officinale onto the dendrimers were known by the UV-Visible spectroscopy (Beckman). Infrared spectra were recorded on a Fourier transform infrared (FT-IR) spectroscopy (Perkin Elmer Spectrum RX 1)

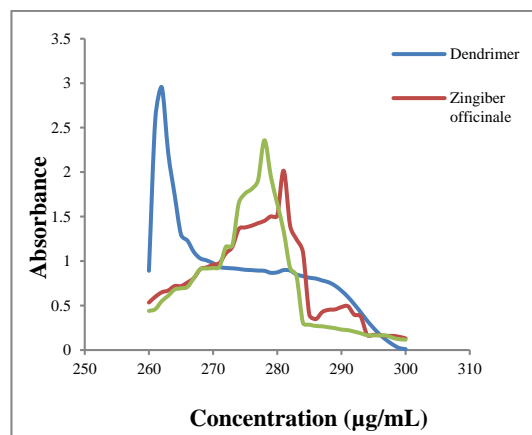


Figure 3: Comparison of the UV-Vis spectrum

using the potassium bromide (KBr) pellet method in the range of 4000-400 cm⁻¹. The different spectra were generated.

Anti cancer activity

MTT assay

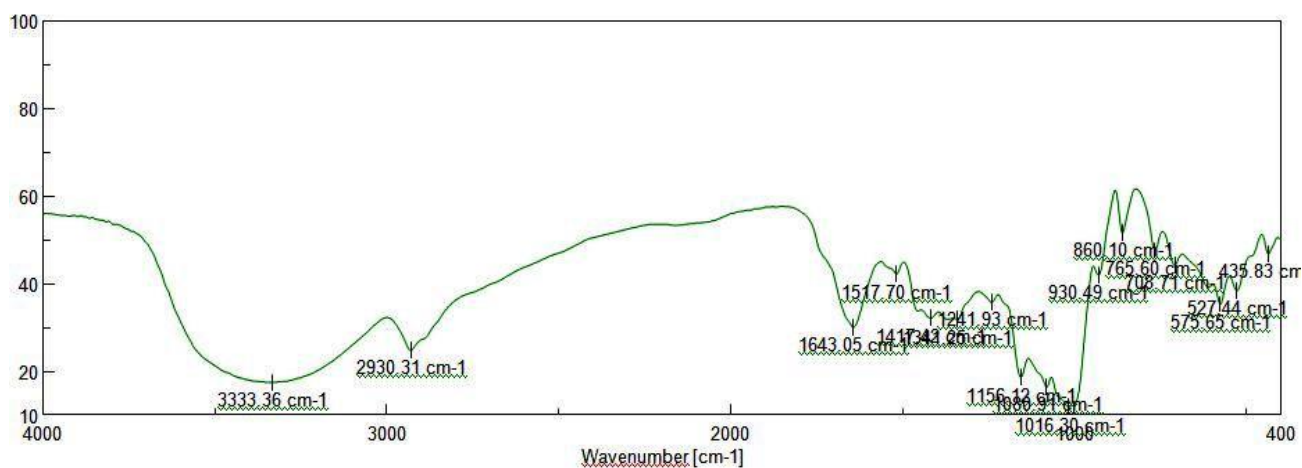
To evaluate the anticancer activity of Dendrimer, *zingiber officinale* and DNZ's using MTT assay protocol by PlumbJA¹⁵

The HaCaT and HEp2 cells at 3 x 10⁵ cells/well were seeded in a 96 well plate. The plate was incubated overnight at 37°C in a humidified incubator, 5% CO₂ for the normal and cancer cells to grow and adhere to the surface. Test compounds (Dendrimer, *zingiber officinale*, DNZ's) were added to the plate. Include replicates for a range of concentrations. Include negative controls (including vehicle control) and a positive control. The final volume will be 100µl per well. The plate was incubated for overnight (or for some other appropriate time) at 37°C in a humidified incubator, 5% CO₂. MTT reagent (20µl/100µl per well of the 96 well plate) was added and incubated at 37 °C for 3 hours. 1 volume (100µl) of the stop mix solution was added and the plate was rocked at room temperature for a minimum of 1 hour. A purple colour should be visible at this stage and should deepen over the 1 hour incubation period. After the 1 hour incubation, ensure the formazan precipitate is dissolved by pipetting each well up and down until no precipitate is visible. Read the plate on a plate reader using wavelength at 572 nm.

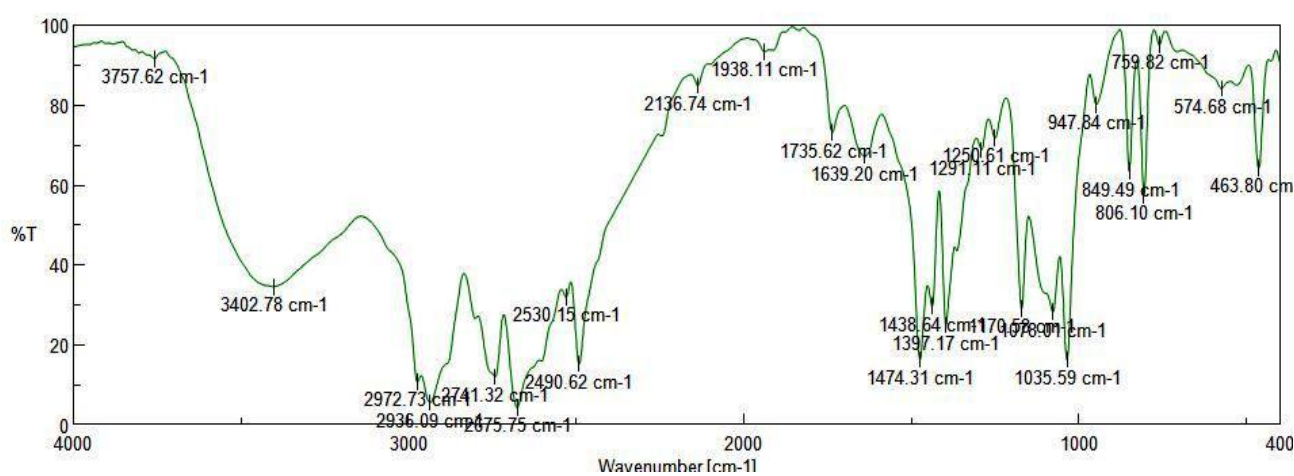
RESULTS AND DISCUSSION

UV-Vis Spectroscopy

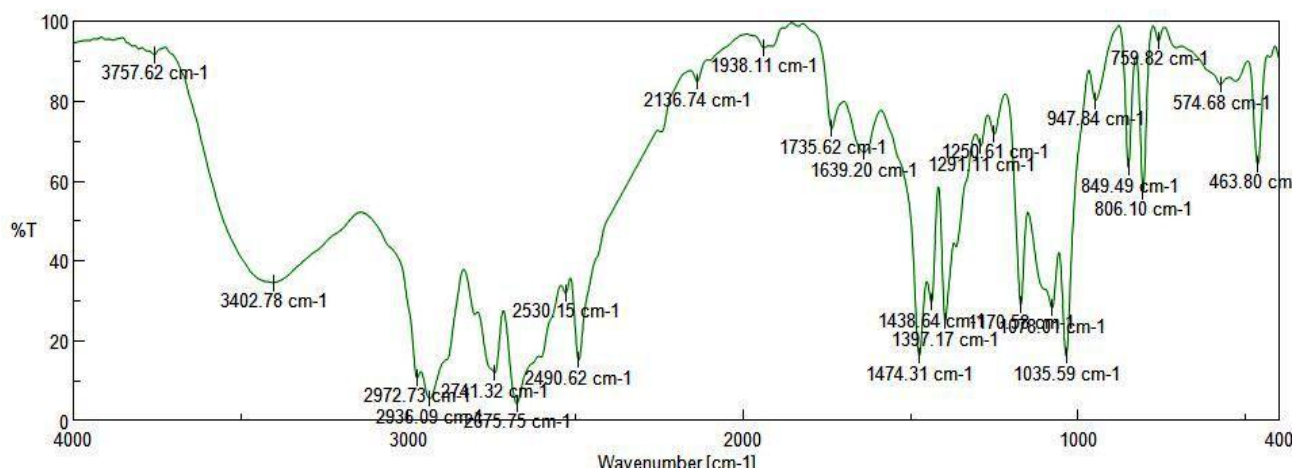
Figure 3 shows the UV-Vis spectrum of zingiber officinale, dendrimer and dendrimer- zingiber officinale. The absorbance peaks were recorded using the UV-Visible Spectroscopy. The absorbance peak was found to be at 262 nm for the naked dendrimers. zingiber officinale showed the characteristic peak at 281 nm. There was a shift in the peak from 262nm to 278nm for the zingiber officinale bound dendrimers confirming the successful binding of zingiber officinale onto the dendrimer.



a



b



c

Figure 4: FT-IR spectra of *zingiber officinale* (a), DNZ's (b) and Dendrimer (c).

FT-IR analysis

Figure 4 shows the fourier transform infrared spectrum of *zingiber officinale* (a), DNZ's (b) and Dendrimer (c). FT-IR technique is used to confirm the functional group present in dendrimer as well as the binding of *zingiber officinale* on dendrimer. The major infrared bands of *zingiber officinale* at 3333, 2930, 1643, 1016, 1341 cm^{-1} which is attributed to O-H(H bonding), C-H, C=C, C-O, C-H

stretching frequency respectively. The bands of Dendrimer at 3414, 2936, 1643, 1035, 1397 cm^{-1} are assigned to stretching of O-H(H bonding), C-H, C=C, C-O, C-H functional groups. The spectral shifting was observed for DNZ's at 2936, 1639, 1035, 1397 cm^{-1} . This shows the confirmation of *zingiber officinale* bound Dendrimer.

MTT Assay

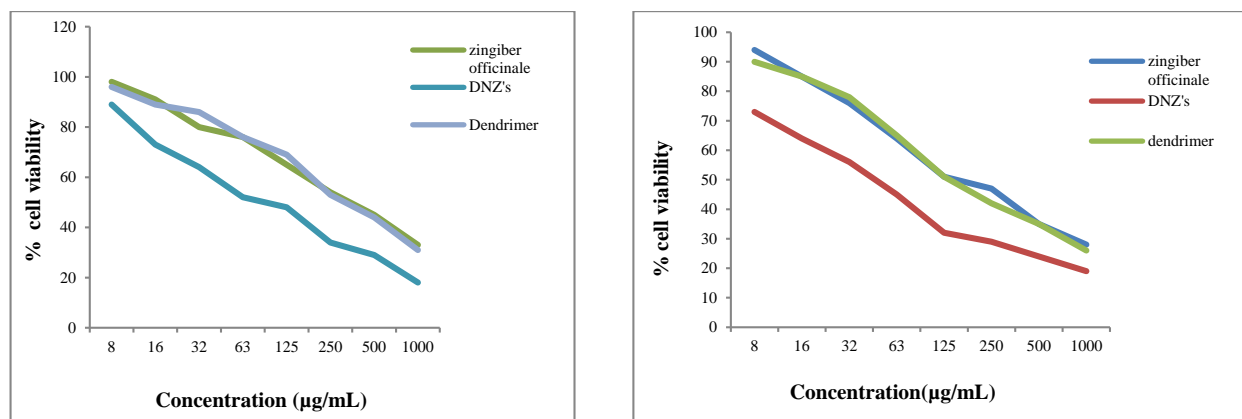


Figure 5: % cell viability for 48 hrs drug incubated wells (a) HaCaT Cells (b) HEP 2 Cells

The MTT assay results for 48hrs incubation of test

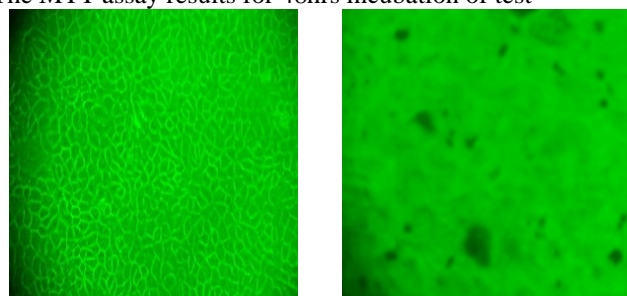
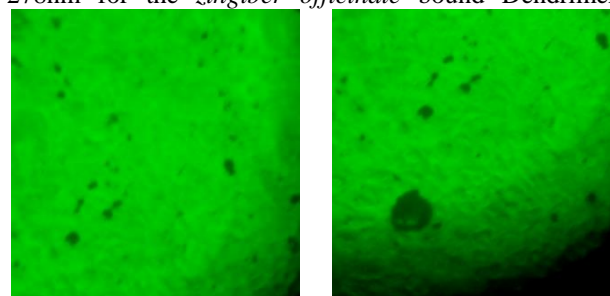


Figure 6: IC₅₀ of untreated (a) and treated with Dendrimer (b),

278nm for the *zingiber officinale* bound Dendrimers



Zingiber officinale (c) and DNZ (d) HaCaT cell lines

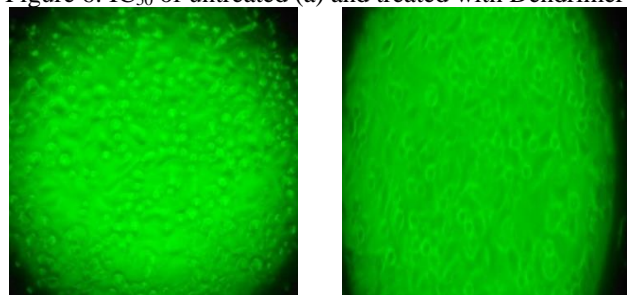
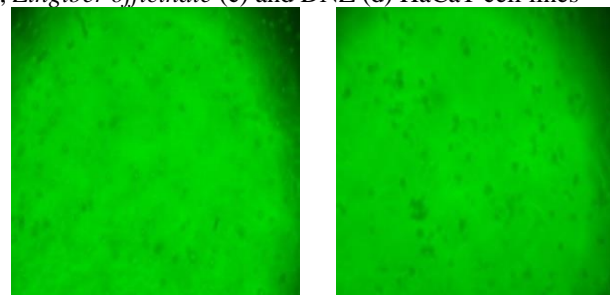


Figure 7: IC₅₀ of untreated (a) and treated with Dendrimer (b),



compound (naked Dendrimer, naked *zingiber officinale*, DNZ's) such as at the concentrations of (1000-8µg) showed increased HaCaT and HEP2 cell viability as the concentration got diluted.

The results shows that Median IC₅₀ (concentration that inhibits cell growth by 50%) values for anticancer activity of the naked *zingiber officinale*, Dendrimer were 250µg/ml for normal cell lines (HaCaT) and 125µg/ml for cancer cell line (HEp 2). *zingiber officinale* bound Dendrimer(DNZ's) were 63 µg/ml for normal cell line (HaCaT) and 32 µg/ml for cancer cell line (HEp 2). This shows that DNZ's has high anticancer activity compared to naked Dendrimers and *zingiber officinale*. At low concentration, DNZ has higher activity against cancer cells.

CONCLUSION

In summary, the Dendrimer structure were synthesized and characterized by UV visible spectroscopy and FT-IR spectroscopy. There was a shift in the peak from 262nm to

confirming the successful binding of *zingiber officinale* onto the Dendrimer. In FT-IR, the spectral shifting was observed for DNZ's at 2936, 1639, 1035, 1397 cm⁻¹ which confirms the *zingiber officinale* bound Dendrimer. The IC₅₀ value of DNZ's was 63 µg/ml for normal cell line (HaCaT) and 32 µg/ml for cancer cell line (HEP 2). This shows that DNZ's has high anticancer activity compared to naked Dendrimers and *zingiber officinale*. From this study, we conclude the higher activity of DNZ at low concentration helps for further exploitation in drug discovery and clinical applications.

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