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# Antifungal activity of neem and *Aloe vera* formulation mediated zirconium oxide nanoparticles

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ABSTRACT

The aim of the research is to access the Anti-fungal activity of Neem and *Aloe Vera* composition mediated zirconium oxide nano particles. The procedure includes a collection of *Azadirachta indica* (neem) and *Aloe Vera* plant leaves and preparation of plant extract, Synthesis of zirconium oxide nanoparticles using a herbal formulation and Anti-fungal activity determination of synthesised zirconium oxide nanoparticles. The objective is to evaluate the activity done by neem and *Aloe Vera* composition mediated zirconium oxide nano particles against fungal infection. As a result, the addition of nanoparticles to prepared plant extracts showed mild or no colour change. Thus zirconium oxide nanoparticles are colourless in nature. But turbidity in solution appears after the addition of nanoparticles. Graphical representation of the synthesis of nanoparticles showed a peak at 350 nanometers(nm) in both initial and final readings, but the rate of absorbance showed a mild increase in final reading which confirms the synthesis of the nanoparticle. The anti-fungal activity was checked against 3 pathogenic fungi *Aspergillus fumigatus*, *Aspergillus niger, Candida albicans* and it showed mild ring of a zone of inhibition around the well. Zone of inhibition increases with an increase in the quantity of nanoparticles in the well. Zone of inhibition proves the anti-fungal activity. To conclude, *Azadirachta indica* (neem) and *Aloe Vera* composition mediated zirconium oxide nanoparticles show anti-fungal activity.

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## INTRODUCTION

Nanotechnology can be explained as the manipulation of matter on the molecular, atomic and supramolecular scale. The earliest explanation of nanotechnology referred to the particular technological goal of precisely manipulating molecules and atoms for the fabrication of nanoscale products. Nanotechnology can also be called as molecular nanotechnology (Rajeshkumar and Bharath, 2017; Santhoshkumar *et al.*, 2017). Nanoparticles are particles which have their size ranges between 100 and 1 nanometers (nm) in size which is surrounded by an interfacial layer. The interfacial layer is an essential part of nanoscale matter, which basically affect all of its properties. The interfacial layer consists of organic molecules, ions and inorganic molecules. Organic molecules coating inorganic nanoparticles are known as stabilisers, capping, and surface ligands, or passivating agents (Rajeshkumar and Naik, 2018). In nanotechnology, a particle is cited to as

a small object which behaves as a whole unit with respect to its transport and properties. Particles are further classified according to diameter. Nanoparticles can be classified into different types based on their size, morphology, chemical properties and physical properties. Some of the types of nanoparticles are ceramic nanoparticles, metal nanoparticles, carbon-based nanoparticles, semiconductor nanoparticles, polymeric nanoparticles and lipid-based nanoparticles (Agarwal *et al.*, 2018; Menon *et al.*, 2018).

Zirconium oxide nanoparticle is a metal oxide-based nanoparticle. Zirconium oxide nanoparticles (ZrO2) are present in the forms of nano-fluids and nanocrystals and nanodots having a white surface area. Zirconium oxide nanoparticles are often doped with yttrium oxide, calcia or magnesia. Zirconium is a Block D, Period 5 element and oxygen is a Block P, Period 2 element. Zirconium oxide is also known as, zirconium, zirconic anhydride, zirconia and zircol. In our research, we are going to see Antifungal activity of neem and aloe vera composition mediated zirconium oxide nano particles: *Azadirachta indica,* binomial name of neem.

Neem is also called as nimtree, which is a tree in the mahogany family Meliaceae. It is one of two species in the genus Azadirachta and is native to the Indian subcontinent, which includes countries like India, Pakistan, Nepal, Sri Lanka, Bangladesh, Maldives and Myanmar. Neem is usually grown in tropical and sub-tropical regions. Neem trees are also grown in some islands located in the southern part of Iran. Neem seeds and fruits are the main source of neem oil. *Aloe vera* is a fleshy plant species grouped under the genus Aloe. *Aloe Vera* is a perennial, evergreen plant which got its origin from the Peninsula of Arabia but usually grows wild in tropical climates all around the world. Aloe Vera is basically cultivated for agricultural purposes and medicinal uses. *Aloe Vera* can also be used for decorative purposes in pots as they grow successfully in indoors (Upadhyay, 2018).

In our research, we are going to use neem and *Aloe vera* with zirconium oxide nanoparticle to check their antifungal activity. Drugs derived from natural sources, i.e. herbal source, play an important function in the treatment and prevention of disease, which affects human beings. In most of the developing countries like India, Brazil, traditional medicine is believed to be one of the primary healthcare systems. About 60% - 65% of new drugs which was developed between years of 1980 and 2002 made use of natural products, i.e. herbal products as one of the raw material and they are very successful even now, specifically in the zones of infectious disease caused by bacteria, fungi, etc. and also effective in treating cancer (Cragg and Newman, 2005).

Current trends, however, show that the discovery rate of active novel chemical entities are dropping due to adverse effects caused by chemicals (Lam, 2007) Natural products prepared from plants may give a new source of antimicrobial/antifungal agents with successful novel mechanisms of action (Bonjar, 2004; Runyoro *et al.*, 2006). Apart from its pharmacological uses, the plant extract basically which are herbal products are also prescribed as a pest and disease control agents in developing countries like Brazil, India (Jaipal *et al.*, 1983; Sharma and Basandrai, 1999; Raja *et al.*, 2000). Neem and Aloe Vera plants are widely used by tribal people and even by people who live in villages use to treat various infections including ringworm and other fungal skin infections (Rajan *et al.*, 2001).

## MATERIALS AND METHODS

### Collection and preparation of plant extracts

The healthy and fresh leaves of plants *Azadirachta indica* (neem) and *Aloe Vera* were collected from September to December 2018 from various areas of Chennai, Tamil Nadu, India. The collected leaves, i.e. neem leaves and Aloe Vera leaves, were washed for 3-4 times using distilled water. The washed leaves were dried in the shade for 7-14 days. The well-dried leaves of neem and Aloe Vera were picked and made into powder by using mortar and pestle separately. The collected powder was stored the separately airtight container with 100% relative to humidity. 1 gram of *Azadirachta indica* (neem) powder and 1 gram of *Aloe vera* powder was weighed and was dissolved in 100ml of distilled water and boiled for 5-10 minutes at 60 degrees to 70 degrees Celsius. The solution was filtered by using Whatmann's No.1 filter paper. The filtered extract was collected and stored in 4 degree Celsius for further use.

### Antifungal activity

### Synthesis of nanoparticles

10 millimolar of Zirconium oxychloride octahydrate 98% is prepared in 90 ml of double-distilled water. Plant extracts of *Azadirachta indica* (neem) and *Aloe Vera* is added with a metal solution and was made into 100 ml solution. The prepared solution is poured into a conical flask, labeled and then kept in an orbital shaker for nanoparticle synthesis.

### Characterisation of nanoparticles

The synthesised nanoparticle solution is preliminar-


**Figure 1: Visual observation Plant extract and Nanoparticles**

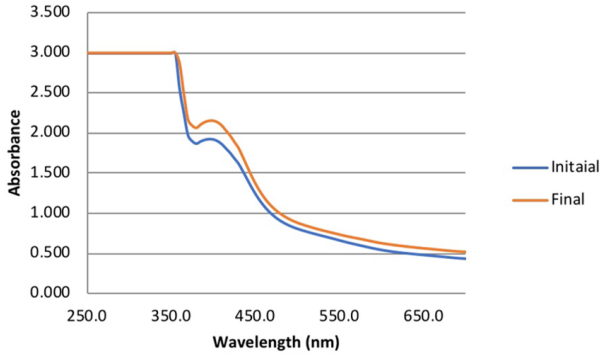


**Figure 2: UV-vis spectroscopy**

ily characterised by using UV-vis-Spectroscopy. 3ml of the solution is taken in curettes and scanned in double beam UV-vis-Spectrometer from 250nm – 700nm wavelengths. The results were recorded for graphical analysis.

### Preparation of nanoparticles powder

The nanoparticles solution is centrifuged using refrigerated lark centrifuge. The solution with Zirconium oxychloride octahydrate 98% nanoparticles is centrifuged using centrifuge tube and kept in lark refrigerated centrifuge at 8000 rpm (rotations per minute) for 10 minutes, and the pellet is collected and washed with distilled water twice. The final purified pellet is collected and dried at 110 degree Celsius for Zirconium oxychloride octahydrate 98% nanoparticles for 20-24 hours. Finally, the nanoparticles powder is collected and stored in airtight Eppendorf tub.

### Antifungal activity

Antifungal activity of the nanoparticles carried out based on our previous research work

## RESULTS AND DISCUSSION

Antifungal activities of *Azadirachta indica* (neem) and *Aloe Vera* mediated zirconium nanoparticles examined. Antifungal activities of plant part extract against three pathogenic fungi were investigated by the agar disk diffusion method (Rajeshkumar *et al.*, 2014; Rajeshkumar, 2016; Karthiga *et al.*, 2018). On visual observation colour of solution remains the same before and after adding Zirconium oxychloride octahydrate 98% nanoparticles. But the solution becomes turbid after adding Zirconium oxychloride octahydrate 98% nanoparticles (Figure 1).

### UV-vis spectroscopy

UV-Vis Spectroscopy refers to reflectance spectroscopy or absorption spectroscopy in the ultraviolet-visible spectral region. This means it uses light in the visible and adjacent ranges. UV-vis Spectroscopy is carried out using UV-vis Spectrometer.

On Figure 2, initial representation analysis of the

 

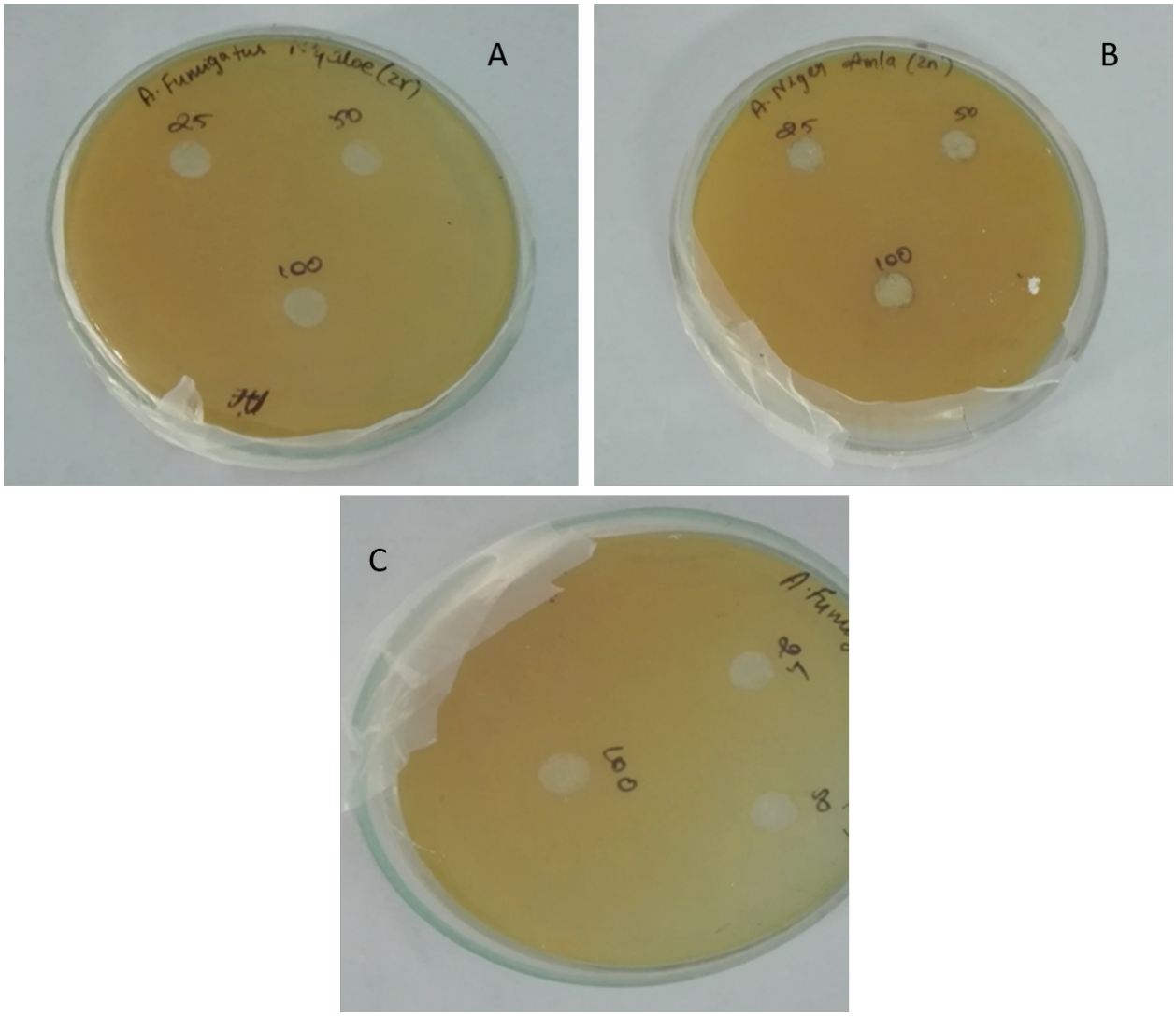


**Figure 3: Antifungal activity of Zirconium oxide nanoparticles (a) Aspergillus fumigates (b)Aspergillus niger (c) Candida albicans**

synthesis of nanoparticles showed a peak at 350 nanometers(nm). The final analysis also showed a peak at 350 nanometers (nm), but the absorbance rate is quite more in case of the final analysis, which depicts that nanoparticles are completely synthesised.

For finding anti-fungal activity Rose Bengal agar was inoculated with colonies of pathogenic fungi *Aspergillus fumigatus, Aspergillus niger* and *Candida albicans.* Another nutrient agar medium was inoculated pathogenic fungi *Candida albicans* and well was formed.

In each and every medium 3 wells were formed. Each well was labled. There is a mild zone of inhibition observed in all 3 medium and thickness varies with an increase in added quantities of nanoparticles (Figure 3). Thus, it shows anti-fungal activity against pathogenic fungi.

## CONCLUSION

In the current investigation, we made use of *Azadirachta indica* (neem) and *Aloe Vera* plant extracts mediated with the addition of zirconium oxide nanoparticles to check the anti-fungal activity. To check the anti-fungal activity prepared nanoparticle solution was introduced to pathogenic fungi *Aspergillus fumigatus, Aspergillus niger*, *Candida albicans*. It forms a thin ring-like zone of inhibition around the well. Zone of inhibition increases with an increase in the quantity of nanoparticle. Zone of inhibition proves that there is an anti-fungal activity shown by *Azadirachta indica* (neem) and *Aloe Vera* mediated zirconium oxide nanoparticles.

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