ACTA SCIENTIFIC AGRICULTURE (ISSN: 2581-365X)

Volume 3 Issue 6 June 2019

Research Article

Synergistic Effect of Silver Nanoparticles Produced by Green Synthesis and Neem Oil (*Azadirachta Indica*) Against Human Pathogenic *Candida Albicans*

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Received: March 21, 2019; **Published:** May 22, 2019

DOI: 10.31080/ASAG.2019.03.0491

Abstract

Background: Neem oils (NO) and silver nanoparticles (AgNPs) are different substances. However, both have been studied due to its antibacterial, antifungal and antiviral properties.

Aim: The justification of work was evaluating the synergistic activity of neem oil (*Azadirachta indica*) associated with silver nanoparticles (AgNPs) against human pathogenic *Candida albicans* (*C. albicans*).

Methods: The neem oil was extracted from the seeds of *Azadirachta indica*, while the AgNPs were synthesized, purified and characterized by using non-toxic compounds (glucose and ribose). For this study, 10 strains of *C. albicans* were collected, purified and identified. Synergism between the neem oil and the AgNPs was evaluated by the disk diffusion method.

Results: The AgNPs had adiameter of 98 ± 60.32 nm (mean ± SD) for those produced from glucose and ribose. The main constituents of the neem oil were nimbin, nimbidinin, azadirachtin, nimbolide. The AgNPs prepared by using glucose and ribose brought about an increase in the fungal growth inhibition, correspondingly, compared to the oil used.

Conclusion: Neem oil acts by destabilizing fungal cell membrane and AgNPs inactivate the DNA, enzymes and other cellular components and prevent *C. albicans* replication, it leads to death. This innovative combination (neem oil + AgNPs) has potential to be used in the treatment of *C. albicans* and other fungal infections. It can also used as an alternative product from renewable source.

Keywords: Neem Oil; AgNPs; SEM; C. albicans; Antifungal Activity

Abbreviations

NO: Neem Oils; AgNPs: Silver Nanoparticles; *C. albicans: Candida albicans*

Introduction

C. albicans is an emerging multidrug-resistant fungal pathogen representing an important source of invasive disease in humans and generating high healthcare costs worldwide. Present over all 150 spp. of Candida fungi, of which around 20 are acknowledged to be pathogenic to human beings [1]. *Candida* spp. is frequently found in different anatomical sites of healthy persons and could provoke systemic and superficial infections under most favorable environmental situations [2]. Candida normally lives in the mouth, throat, and the rest of the digestive tract without causing any problems. Sometimes, *Candida* can multiply and cause an infection if the environment inside the mouth, throat, or esophagus changes in a way that encourages its growth. This can happen when a person's immune system becomes weakened. [3]. Plants have been an important source of natural products for maintaining human health. According to WHO medicinal plants would be the best source to obtain a variety of active drug molecules. Most of people use traditional medicines that have been obtained from medicinal plants. Current and traditional systems of medicines, the medicinal plants continue to serve as valuable therapeutic agents [4]. *A. indica* widely used as a medicine in different fields of medicine [5]. Nanotechnology is a field of science that is growing and bringing together

various fields, such as Pharmacy, biology, engineering, physics and chemistry. Particles sizes range from 1 to 100 nm [6]. The composition of a nanomaterial plays a vital role in determining its biological interactions [7]. Nanoengineering has led to strong interest in how nanomaterials interact with biological systems [8].

Materials and Methods

Synthesis of Ag-NPs

For green synthesis of Ag-NPs, 1.0 g of glucose and sucrose was added to 100 mL of H_2O in a flask, and the solution was stirred to obtain a clear solution. To synthesis the Ag+/ glucose and sucrose solution, the silver solution (10 mL, 1 M) was added to the glucose and sucrose solution with continuous stirring. The above solution was distributed into three different cuvettes, and the prepared solutions were stirred continuously at room temperature until change the colour. All solutions were stored in dark place to avoid photochemical reactions during the experiment.

Collection of fungal cultures

For this study, 10 strains of *C. albicans* were collected from Sree Abirami multi-specialty hospital. From these 10 strains, most resistant strain selected for antifungal and synergistic activity.

Antifungal activity

Antifungal activity of Ag-NPs and Neem Oil were studied by disc diffusion method. Resistant *C. albicans* fungal strain was used for screening the antifungal activity. *C. albicans* inoculum was prepared by using potato dextrose broth. PDA media were prepared by autoclaving 3.9 g in 100 ml. Preparation of *C. albicans* mat on the PDA petri plates using sterile cotton swabs. Ag-NPs and Neem oil were placed on sterile discs, which were dried aseptically under laminar air flow to remove the solvent. The dried discs were placed on the surface of culture inoculated PDA plates and the plates were incubated at room temperature for 48 h. Antifungal activity was evaluated by HiMedia zone reader.

Results and Discussion

The AgNPs had a diameter of 98 ± 60.32 nm (mean ± SD) for those produced from glucose and ribose (Figure 1, 2). The main constituents of the neem oil were nimbin, nimbidinin, azadirachtin, nimbolide. The AgNPs prepared by using glucose and ribose brought about an increase in the fungal growth inhibition, correspondingly, compared to the oil used (Figure 3, Table 1). Neem oil acts by destabilizing fungal cell membrane and AgNPs inactivate the DNA, enzymes and other cellular components and prevent *C. albicans* replication, it leads to death [9].

Figure 1: Glucose and sucrose Ag-NPs.

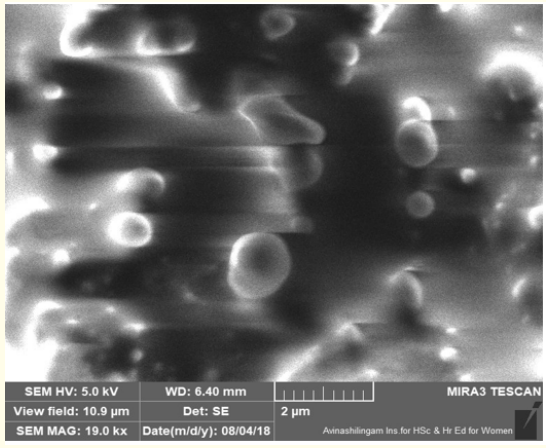

Figure 2: FE SEM image of Nanoparticles.

Figure 3: Effect of Neem oil and AgNPs against to *C. albicans.*

S. No.	Name of substance	Microorganism	Zone of Inhibition (mm)
1	Neem + AgNPs	*C. albicans*	20mm

Table 1: Antifungal activity of Neem oil + AgNPs.

Conclusion

Neem oil acts by destabilizing fungal cell membrane and AgNPs inactivate the DNA, enzymes and other cellular components and prevent *C. albicans* replication, it leads to death. This innovative combination (neem oil + AgNPs) has potential to be used in the treatment of *C. albicans* and other fungal infections. It can also used as an alternative product from renewable source.

Acknowledgements

Author is thankful to department of Pharmaceutical Technology, Anna University, BIT campus, Tiruchirappalli, for providing all facilities necessary to carry out the work.

Conflict of Interest

No conflict of interest

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