Available online at www.elixirpublishers.com (Elixir International Journal)

Bio Sciences

Elixir Bio Sci 116 (2018) 50269-50272

ISSN: 2229-712X

Anti Candidal Activity of the Green Synthesized Silver Nano Particles against Candida Species Isolated from Onychomycosis

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ARTICLE INFO

Article history:

Received: 10 February 2018;

Received in revised form:

5 March 2018;

Accepted: 17 March 2018;

Keywords

Green synthesis,

Silver nanoparticles,

Candida,

Onychomycosis.

ABSTRACT

The impacts of superficial mycosis badly affecting the individuals.The nails, hair and skin infections collectively known as dermatophytosis and its creating a social stigma. Both dermatophytes and non dermatophytes are the cause for the nail infections but onychomycosis is a non dermatophytic nail infection. Considerable percentage of the onychomycosis is caused by the candida species .Drug resistant and multi drug resistant candida often isolated from the onychomycosis which interferes in treatment. In view of finding the effective solution for these drug resistant candida strain,The present study was undertaken. Totally 30 numbers of the candida isolates which were originally isolated from the onychomycosis cases were subjected to the antifungal activity of the green synthesized silver nano particles. *Terminalia chebula* was used in green nano synthesis and reduced silver nitrate bulk materials to nano size materials. It was recorded that the green synthesized silver nanoparticles comparatively shown their best anti candidal activity than aqueous extract of *T.chebula* and $AgNo_3$ aqueous solution.

Introduction

Onychomycosis has been traditionally referred to a nondermatophytic infection of the nail but currently used as a general terminology to denote nail infection caused by any fungi (Weitzman et al.,., 1995). Medical research information indicate the fact of onychomycosis and its significant negative impacts on patient's emotional, occupational and social life style. The ultimate social stigma, put them in fear and lead to diminished self-esteem which in turn, the patients tend to avoid even their close relationship (Scher 1963). Though there exist variability within the fungi,that are pathogenic to onychomycosis, this disease is mainly and predominantly caused by dermatophytes. Compared to the other fungal etiology involvement in onychomycosis candida species recorded with considerable percentage (%).

Nanoparticles ranges the sizes between 1-100 nm (Rai et al.,2009,EU 2011,Adlakha-Hutcheon et al.,2009).Nano technology is described as a combining or involving several academic disciplines of science, consists of different aspects of research and technology (Uskokovic, 2008). Nanoparticles have wide variety of potential application in biomedical, optical and electronic fields. The history of nanoparticles and its uses dates back to the 9th century in Mesopotamia where the artisans used this nanoparticles to generate a glittering effect on the surfaces.Silver nanoparticles are used in biomedicine (Jonghoon Choi and Nam Sun Wang 2011).

The resent and retrospective research publications are signifying the importance of biological methods of synthesizing nanoparticles, which attracted the different fields of chemical electronic and the biological science.The impacts created by the nanoparticles in nanotechnology become more significant at global level.Nanoparticle research has been increasingly studied by the various fields of life science including biology and medicine. Because of their unique and significant properties, nanoparticles are highly influenced.

In medicine, the uses and advantages of nanoparticles mixed-up in creating florescent biological labels for constant biological markers and molecules in research and diagnosis of diseases, drug delivering systems, gene delivering system in gene therapy, for biological detection of disease causing organisms and diagnosis detection of proteins isolation and purification of biological molecules and cells,probing of DNA structure, genetic and tissue engineering, destruction of tumors with drugs or heat in MRI study and pharmacokinetic studies(Bruchez et al., 2013;Chan,Nie 2016;Wang et al., 2002;Mah et al.,2000;Panatarotto et al., 2003; Edelstein et al., 2000; Nam, et al., 2003; Mahtab R et al., 1995; Ma, et al., 2003; de la Isla,et al.,2003; Yoshida J et al., 1999; Molday, MacKenzie 1982 and Weissleder, et al., 1990).

Nanoparticles have its advantage in drugs delivery system because of their size and surface characteristics which can be easily manipulated.Nanoparticles can be used to control and sustain release of the drugs as well as subsequent clearance of the drug from the body, can be altered the drug therapeutic efficiency and reduction in side effects.By using this nanoparticles,targeted drugs may be developed and this drugs can be administered through various roots such as oral, nasal, intraocular or parenteral. Research reports are available about the biosynthesis of Silver,Gold, alloy,Telliurinium, Platinum, Palladium, Silica, Titanium, Magnetic and Uranite nanoparticles by bacteria,fungi and viruses.To increase the rate of biological nanoparticles, it is essential to mediate the synthesis of biological nanoparticles and that should be studied in detailed manner at cellular biochemical and molecular mechanism level.

Though several techniques such as ultraviolet radiation, aerosol techniques, lithiography, laser ablation,ultra semi field, and photo chemical reduction techniques have been so far successfully used to produce nanoparticles, these expensive techniques involve the use of hazardous chemicals. In view of developing the eco - friendly and sustainable method, in this study, we have used the green synthesis method in silver nano synthesis.Many researchers had been published about the bio synthesis of nanoparticles which includes green synthesis of nanoparticles in which the plants are used to synthesis the nanoparticles and the microbial nano synthesis method where the microorganisms have been used. The biosynthesis method of nanoparticles production is a green chemistry approach that inter connects the field of Nanotechnology, Microbiology and Biotechnology.

Metal nanoparticles such as silver, gold, platinum, magnesium etc. have obtained appreciable attention at global level in recent times because of their basic and technological interest.If overview the most recent developments in bio medicine and other scientific field of research the nanoparticles research increasingly attracted the interest of the researchers which in results with effective applications of nanoparticles.These nanometer sized objects, entered a commercial exploration field.

In view of interconnecting the herbal science, green chemistry,nanoscience, medical mycology and medicine, the present study was undertaken to synthesize silver nanoparticles by green synthesis and study its anticandidal activity against the candida species isolated from the onychomycosis.

Material and Methods

Specimen source and clinical specimens

The clinically suspected cases of onychomycosis are the sources of the respective clinical specimens. The infected nail specimens were collected in a sterile glass containers,to ensure the fungal etiology.Specimens were collected from the active site of infection to maintain the accuracy in the diagnosis. Three different specimens from the same site of the infection at three different days from each patient had been collected to make it confirm the association of the respective fungi in the onycomycotic cases. All the collected specimens from the site of collection were promptly transported within two hours from the time of collection with proper labeling for further direct microscopic study.For candida culture, spot inoculation was performed. For this, from the collected nail specimen 2 or 3 fine piece of nail was inoculated directly on SDA with antibiotic, at the site of specimen collection.

Direct Microscopic examination of the nail specimens

All the nail specimens collected from onychomycosis cases were subjected to the direct microscopic examination and isolation of candida. Direct microscopic examination of the nail specimen was performed with 20 % KOH wet mount examination. Presence of budding yeast cells with or without pseudohyphae in the nail specimen was examined and the results were recorded.

Mycology Culture

Candida isolation

The nail specimens collected from the onychomycosis cases were immediately inoculated on SDA with anti different types of the superficial infections were inoculated separately on SDA agar containing antibiotics to prevent the bacteria present in the specimens.Each specimen was inoculated on to one set of SDA plates. One plate was incubated in BOD incubator at 25°C and another was incubated at 37°C for maximum three days. Fungal growth was observed from 24 hours onwards till third day. Fungal culture negative results were issued after third day when there was no visible growth of candida.

Identification of fungal species

Candida was identified by the traditional laboratory procedures such as macroscopic and microscopic examination of fungal culture.In addition certain bio- chemical reactions and certain confirmatory tests for candida, such as sugar fermentation, sugar assimilation,urease test,germ tube formation, chlamydospore formation, was used in the candida identification and speciation .

Green synthesis of silver nano particles

The south Indian medicinal plant, *T. chebula* whole fruit extracts (aqueous),freshly prepared and used in the green nanosynthesis of bulk silver nitrate materials.For this test tubes contained 3ml of the aqueous solution (0.1mm) of $AgNo_3$, was slowly added with 0.5 ml of freshly prepared aqueous extract of *T.chebula* for nanosynthesis.The content was manually shaken and watched for the visual colour change which is considered as one of the typical characteristics of nano production. Further the content was filtered through sterile Whatman No.1 filter paper.The content from the filter paper was gently and carefully taken out and placed in the sterile glass plate.This was placed in the hot air oven at 60 degree centigrade till it get completely dried.Then that was macerated.The powder was subjected for further test procedures.The dark materials adhered on the glass container was washed with acetone and that was also subjected for the anticandidal activity.

Anti candidal activity test

Agar well diffusion method was followed to study the anticandidal activity of the green synthesized silver nanoparticles.The molten sabourauds dextrose agar without antibiotic was poured in to sterile petri dishes and allowed for solidification.After solidified,10 micro liters of the standardized candida inoculum (Mac Farland No. 0.5) was delivered to the agar plate.With the help of the sterile swab, that was uniformly spread on the SDA, by rotating the plate while inoculation.After 6mm wells were made on already,inoculated plates.The specified quantity(60 micro liters) of the test agents i.e.*T.chebula* aqueous extract,silver nitarte aqueous solution,green synthesized silver nano particles,and the dark adhered acetone washed material after nanosynthesis were carefully delivered to the cut wells separately and incubated at 37 degree centigrade for 48 hours. The zone of inhibition formed around the wells were measured and recorded.

Results and Discussion

The aim of our study was to synthesize silver nanoparticles by green synthesis method and screen its antifungal activity against the candida species isolated from onychomycosis.Like drug resistant bacterial infections, fungal strains also emerge with resistance to the existing antifungal drugs.The drug resistant fungal infections are documented by many authors.The pathogenic fungal strains not only exhibiting resistance single antifungal drug but also exhibiting resistance to more than one drug. In our study, we could isolate the drug resistant candida and found to be sensitive to the green synthesized silver nanoparticles (Fig. 5 a & b).

The properties of nanoscale level particles are significantly differencing from their bulk materials properties (Mansoori, 2005).

This author statement is highly acknowledged and our results gaining support. It was possible for us to record the best anticandidal activity of the green synthesized silver nano particles than the aqueous solution of the silver nitrate and *T.chebula* whole fruit extract.

The recent development of nanotechnology showing its tremendous potential applications in the society in various fields,including the major fields such as pharmaceuticals, agriculture, diagnostic and even the medical therapeutics. Since it was proven that the silver nanoparticles' anticandidal property and able to kill/inhibit the fungal cells, the silver nano based antrifungal drugs may be used as good topical applicant which could provide the effective cure for the superficial mycotic infections specially for onychomycosis.

It is very interesting to note that the wonderful anticandidal efficacy expressed by the green synthesized silver nanoparticles against the candida isolates, originally isolated from the onychomycosis.Hence this plant based nanosynthesized silver nanoparticles may be not only used to treat the candida species isolated from onychomycosis, but also can be generally used to treat all types of candida infections.Mahamand Ghannoum 2014,in his research publication,discussed about the period of only chomycosis treatment and its duration dependant antifungal treatment advantages disadvantages. However these author indicating the long duration requirement in the onychomycosis treatment, and the side effects of the respective antifungals.

To avoid such types of side effects caused by the antifungal drugs,and to obtain the fast curing, the effective antifungals without toxic and side effects are highly welcome to the medical society.

Fig. 1. Onychomycosis affected nail.

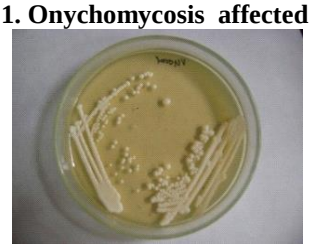

Fig. 2. Candida albicans & *C. kruseii.*

Fig. 3. *C.tropicalis.*

Fig. 4. Antifungal sensitivity Drug resistant candida albicans.

Antifungal activity of silver nanoparticles

Fig. 5a. *C.albicans.*

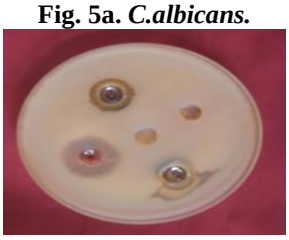

Fig. 5b. *C.tropicalis.*

Note: Wider zone of inhibition formed by Ag nanopartricles than aqueous plant extract and aqueous silver nitrate solution

Silver holds significance since from the olden days. It was used to prevent the spoilage of food and the use of silver spoon and silver vessels are still in practice in our country.In addition to innumerous scientific information about the medical applications, more information yet to be explored regarding the intake of the silver nanoparticles to treat various types of microbial infections.Hence future extensive study in this field of specialization is felt essential.We welcome authors from different geographical area to have collaborative research with us to bringout the complete profile of the green synthesis of silver nanoparticles by using different types of medicinal plants, and their antimicrobial especially antifungal effect.

Based on our research, we can suggest the plant based synthesized silver nanoparticles as the effective remedy which could fasten the cure by express their potential and the extraordinary level of antifungal activity on the fungi involved in Onychomycosis.

Table 1. Susceptibility of Candida Isolates to Green Synthesized Silver Nanoparticles.

S. No.	Test agent	Susceptibility to testing agents		
		***C-albicans* n=15**	***C.tropicalis* n=15**	***C.kruseii* n=15**
1.	*T.Chebula* extract aqueous solution	100 %	100 %	100 %
2.	Silver nitrate aqueous solution	100 %	100 %	100 %
3.	Green synthesized silver nanoparticles	100 %	100 %	100 %

Note: All candida isolates tested for anti-candida activity had shown to be 100% susceptible for all tested agents.

Table 2. Anti-candida Activity of Green Synthesized Silver Nanoparticles (Agar well diffusion).

S. No.	Test agents	ZOI (mm) Minimum & Maximum		
		***C. albicans* n=15**	***C. tropicalis* n=15**	***C. kruseii* n=15**
1.	*T.chebula* extract aqueous solution	10mm – 20mm	12mm – 20mm	9mm – 25mm
2.	Silver nitrate aqueous solution	15mm – 20mm	09mm –13 mm	15mm – 20mm
3.	Green synthesized silver nanoparticles	15mm – 30*mm	20mm – 23*mm	20mm – 25*mm

Note: Compare to plant extract alone and sliver nitrate alone, the green synthesized nanoparticles expressed the maximum anti candida activity (36*mm) equally to three species of candida.

Conclusion

From our study results, we conclude that the *T.Chebula* seed extract based green synthesized sliver nanoparticles expressed its anticandidal activity in different efficacy levels. Compared to the aqueous extract of *T.Chebula* seed and $AgNO_3$, the green synthesized Ag nanoparticles seems to possess the best anti-candida activity.

Hence, the *T.Chebula* seed extract based green synthesized Ag nanoparticles can be either singly or with antifungal combinations used in the treatment of candidacies. We welcome the eco-friendly method of green synthesis, since it is a feasible and innovative and toxic free, than other method of nanosynthesis.

Acknowledgement

We thank our Department Lab-Technicians Mrs. Sumithra and Mrs.Umamaheswari for their high co-operation for their lab assistance. We express our gratitude to Dr. Kabilan, Dean, Faculty of science, Annamalai University to provide the valuable chemicals. We highly acknowledge the suggestions and help rendered by Dr. Krishna Kumar, Professor, Department of Physics, Faculty of Science, Annamalai University, for helping us in nanosynthesis.

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