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Lemon peels mediated synthesis of silver nanoparticles and its antidermatophytic activity

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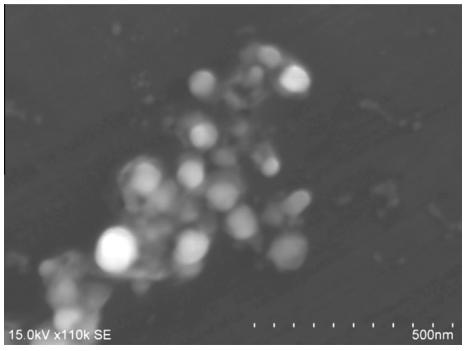
HIGHLIGHTS

- Dermatophytes are isolated from suspected patients, isolated and identified.
- Silver nanoparticles are produced using lemon peels.
- Nanoparticles are characterized using UV, FESEM and EDS analysis.
- Silver nanoparticles were tested for their antimicrobial activity against the dermatophytes.
- Considerable activity was found against the dermatophytes.

GRAPHICAL ABSTRACT

FESEM image of the AgNPs synthesized from lemon peel extract at 500 nm.



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ABSTRACT

There is an increasing commercial demand for nanoparticles due to their wide applicability in various areas. Metallic nanoparticles are traditionally synthesized by wet chemical techniques, where the chemicals used are quite often toxic and flammable. In this work, The extract of lemon peel was prepared and mixed with 1 mM $AgNO_3$ solution .The bioreduction of Ag^+ ion in solution was monitored using UV–visible spectrometer, FESEM and EDAX analysis. Skin scales were collected from patients with suspected dermatophytosis and the dermatophytes were isolated and identified. The AgNPs produced from lemon peels showed good activity against the isolated dermatophytes. The present research work emphasizes the use of lemon peels for the effective synthesize of AgNPs and could be used against the dermatophytes which are found to develop drug resistant towards broad-spectrum antibiotics. The biosynthesis of AgNPs using lemon peel extract is very simple and economic. The use of environmentally benign and renewable plant material offers enormous benefits of eco-friendliness.

Introduction

Nanotechnology is emerging as a rapidly growing field with its application in science and technology [1]. Nanobiotechnology is a field that inter relates both biological sciences and nanotechnology. It provides a platform for the development of ecofriendly and the green synthesis of nanoparticles with the help of biological sources like plants and microorganisms [2]. Various techniques, including chemical and physical means have been developed to prepare metal nanoparticles, such as chemical reduction [3], heat evaporation [4], electrochemical reduction [5] have been reported in the literature. Most of these methods are extremely expensive

Abbreviation: AgNPs, silver nanoparticles.

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and also involve the use of toxic, hazardous chemicals, which may pose potential environmental and biological risks.

Metal nanoparticles biologically synthesized by plant parts like *Punica granatum* peels [6], *Citrus sinensis* peel [7], lemon leaves [8], *Myrica esculenta* leaf [9], *Wrightia tinctoria* leaves [10] has been reported. Using plant for nanoparticles synthesis can be advantageous over other biological processes by eliminating the elaborate process of maintaining cell cultures [11]. It can also be suitably scaled up for large-scale synthesis of nanoparticles. Biosynthesized silver nanoparticles have large number of applications such as in nonlinear optics, spectrally selective coating for solar energy absorption, bio-labeling, intercalation materials for electrical batteries, as optical receptors, catalyst in chemical reactions and as antibacterial capacities [12]. Nanoparticles of silver metal manifest antibiotic activity against bacteria, viruses, and eukaryotes [13–15] and nanosized silver has been used to treat immunologic and inflammatory disease [16]. It is also used for the control of mosquito vectors of malaria, filariasis, and dengue [17].

Dermatophytosis is a superficial fungal infection in keratinized substrates and caused by a group of filamentous fungi called dermatophytes. Among these fungi, *Trichophyton rubrum* is known to account for as many as 69.5% of all dermatophyte infections [18,19]. Several anti-fungal agents including various azoles, tolnafate cream and allylamine derivatives were introduced in the treatment. However, these anti-fungal agents are expensive and have varying degrees of toxicity [20,21]. Hence, there is the need to give greater attention to developing more anti-fungal (anti-dermatophilic) drugs so as to effectively check the increasing prevalence of these infections.

Citrus limon belongs to *Rutaceae* family, common name is lemon and this originated from South East Asia, probably in India or Southern China [22]. It has been used as carminative, insect repellent, antibacterial, larvicidal, antiviral, uricosuric, anti-yeast, antihepatotoxic and antimutagenic agent [23]. Citrus fruits are mainly used by juice processing industries while the peels are generally wasted. Since the juice yield of citrus is less half of the fruit weight, very large amounts of byproduct wastes, such as peels are formed every year [24]. The aim of the study is to use those wasted lemon peels for the synthesis of silver nanoparticles.

Materials and methods

Collection of skin samples

Skin scales were collected from patients with suspected dermatophytosis from the nearest private hospital. The affected area was thoroughly cleaned with 70% alcohol to remove the surface contaminants. Whatman No. 1 filter paper was used for collecting specimens [25]. After disinfection with alcohol, skin lesions were scraped with a scalpel to collect epidermal scales. Collected samples were kept in a sterile container and transferred to the laboratory for further analysis.

Isolation and identification of fungal pathogens

KOH wet mount

Direct microscopic examination was undertaken in 10% potassium hydroxide (KOH) wet mount for the specimens of skin scales for the characteristic macroconidia and microconidia, presence of hyphae and arthroconodia [26,27].

Growth on sabouraud dextrose agar

The KOH positive samples were inoculated in SDA with two different sets of antibiotic incorporated one with chloramphenicol 50 mg/l and the other with cycloheximide 500 mg/l and in addition to chloramphenicol [27]. The culture tubes were incubated at 30 °C for 7–14 days or up to 21 days till the growth appear. Cultures were examined at 4 or 5 days intervals from the onset. After the incubation period the plates were observed and the cultures were subcultured on SDA slants and stored for further characterization. The cultures obtained were also identified on the basis of their macro and microscopic features [28].

Staining with lactophenol cotton blue

The microscopic examination of fungal growth was observed with lactophenol cotton blue staining. The macroscopic examination of dermatophytes was characterized by duration of growth, surface morphology and pigment production on the reverse. Nature of mycelium and conidia formation (macro- and micro-conidia) helps to differentiate various genera and species. Budding yeast cells of *Candida* spp. were identified microscopically. *Candida* species were classified as albicans and non-albicans group by the production of the chlamydospores on corn meal agar and germ tube formation.

Urease test

The ability to hydrolyze urea provides additional data that can be used to aid in the differentiation of *T. rubrum* (urease negative) from *Trichophyton mentagrophytes (T. mentagrophytes)* is typically urease positive [29].

Biosynthesis of silver nanoparticles using lemon peel extract

The lemon peel is washed thoroughly with double distilled water and incised into small pieces. 4 g of thus finely cut *C. limon* were weighed and transferred into 40 ml double distilled water, boiled for 2 min. The extract obtained was filtered through Whatman No. 1 filter paper and the filtrate was collected in 250 ml Erlenmeyer flak and stored at 4 °C for further use. 3 ml of the extract was added to 40 ml of 1 mM $AgNO_3$ solution and kept at room temperature for 5 h.

UV–vis spectra analysis

The reduction of pure Ag^+ ions was monitored by measuring the UV-vis spectrum of the reaction medium at 5 h after diluting a small aliquot of the sample into distilled water and a spectrum was taken on a wavelength from 200 to 600 nm.

FESEM analysis of silver nanoparticles

The morphology of the AgNPs was examined using Field Emission Scanning Electron Microscopy (HITACHI SU6600 FESEM). Thin films of the samples were prepared on aluminium foil by dropping a small amount of the sample and placed on a copper grid, extra solution was removed using a blotting paper and then allowed to dry prior measurements.

Energy dispersive X-ray (edax) analysis

The reduced silver was dried on aluminium foil coated copper grid and EDX analysis of the sample was performed using FESEM (HITACHI SU6600 FESEM) equipped with an EDAX attachment.

Screening of synthesized silver nanoparticles for anti-fungal activity

Standardization of inoculum

Fungal spores were harvested from 7 days old SDA slant culture and washed with 10 ml normal saline in 2% Tween 80 with the aid of glass beads to help in dispersing the spores. The spore suspensions were standardized to 10^5 spores/ml.