Green synthesis of Annona muricata mediated selenium nanoparticles and its antifungal activity against Candida albicans

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ABSTRACT

The emergence of antimicrobial resistance pathogens is threatening public health. Several conventional synthetic drugs are failed to treat resistant pathogens and cause adverse effects, alternative medicines are urgently needed. Recent research in nanomedicines is using our traditional plant materials and increasing their efficacy to treat antimicrobial pathogens. In this study, we have synthesized the Annona muricata-mediated selenium nanoparticles and assessed their antifungal activity against Candida albicans. The common fungi species of C. albicans are associated with various septic infections and oral diseases including oral cancer. In this study, the MIC test result revealed that Annona muricata-mediated selenium nanoparticles significantly inhibit C. albicans growth up to 75% at 100µl concentration. Therefore, AM-SeNPs could be used to treat multiple infections associated with C. albicans. Further research in AM-SeNPs may help bring this medicine to market in the future.

Keywords: Annona, nanoparticles, Candida, muricata

INTRODUCTION

After the COVID-19 pandemic, opportunistic fungi infections are a growing cause of morbidity and mortality worldwide. Especially Candida albicans are one of the most common pathogenic fungi in humans. It is present as a commensal in the human oral cavity, gastrointestinal and vaginal systems, with a high proportion of healthy people carrying it, but it has the potential to become pathogenic under certain situations. Millions of people worldwide are affected by disease symptoms ranging from mild to severe superficial infections of the oral and vaginal mucosae, skin, and nails. C. albicans, on the other hand, cause systemic illnesses with significant mortality rates (1,2). C. albicans mucosal infections are mostly caused by abnormalities in host cellular immunity, including those induced by primary or secondary immunodeficiency, or by alterations in the normal microbiota produced by antibiotic treatment. Infections are also related to defects in epithelial barrier integrity, emphasizing the necessity of intact epithelial function in preventing fungal invasion into the tissue. (3-5). Furthermore, C. albicans infections may be
aided by higher expression of the fungus's virulence characteristics. C. albicans genetic changes that result in phenotypic variability within the fungus have been shown to influence its pathogenicity at epithelial surfaces and systemically. The choice between C. albicans commensalism and illness is thus determined by a delicate balance of fungal virulence and host defense mechanisms (6).

In dentistry, the Candida species cause several infections, due to its strong adherence and biofilm formation, which strongly adhere on the denture surface. Furthermore, Candida species co-aggregates with several plaque bacteria and form the biofilm formation on dentures. Antifungal treatments are useful for treating acute denture stomatitis inflammation; unfortunately, the effectiveness is only short-term, and recurrence is probable quickly after quitting treatment or due to the emergence of drug-resistant strains (7). Therefore, alternative medicine is urgently needed to treat Candida infection. The porous surfaces and hydrophobicity of acrylic resin for denture bases aid in the colonization of C. albicans hyphal forms on the surface. C. albicans is associated with several systemic diseases, the Candida bloodstream infection causes severe septic condition and leads to multiple organ failure (8).

Herbal-based medicines are traditionally used to treat multiple diseases, recently researchers are identifying the potential bioactive compounds from plants to treat anti-microbial resistant pathogens. Due to their lesser negative impact, lower cost, and adverse reactivity to plant treatments as compared to contemporary conventional medications, the use of natural products for the treatment of fungal infections is seen to be an intriguing alternative to synthetic fungicides. India is regarded as a rich source of pharmacological plants, which are primarily employed in preventive and therapeutic medicine (9). The screening of medicinal plants for antimicrobial compounds has grown in popularity, especially the Annona muricata plant possesses multiple beneficial properties in the treatment of multiple human diseases. The tropical fruit plant Annona muricata L., mostly known as soursop, is a member of the Annonaceae family. A. muricata is widely utilized as a folk medicine in India, tropical America, and Africa to treat a wide range of human diseases and disorders, notably diabetes, rheumatism, cancer, and parasite infections (10). Furthermore, recent development in nanotechnology and nanoscience discovers the benefits of green synthesized nanoparticle extracts. Nanoparticles are acts as a carrier, enhancing the plant's bioactive compounds, and less toxic. Selenium is one of the important trace minerals that is required by many mammalian species including humans. It could interact with proteins to function properly in immune systems and regulate thyroid gland activity, thereby reducing cellular damage. Furthermore, because of its antibacterial properties, selenium has been researched as an anti-carcinogenic agent for a variety of cancers (11). Previous research has shown that A. muricata extracts possessed strong antifungal properties against multidrug-resistant Candida albicans (12). However, the antifungal property of Annona muricata-mediated selenium nanoparticles is not established. In this study, we have aimed to synthesize the green synthesis of Annona muricata-mediated selenium nanoparticles and their antifungal activity against Candida albicans.

MATERIAL AND METHODS
Fruit extract preparation
Annona muricata fruits are harvested from species confirmed by a botanical taxonomist. The fruits were washed in sterile distilled water, chopped into small pieces, and dried for several days. Then fried fruit was grinded to make powder, the powder was used to prepare the extract. 10 grams of powdered fruits were dissolved in 100 ml of distilled water and the dissolved solution was heated at 80°C for 3 hrs with continuous stirring. After the extract was filtered using Whatman filter paper and the aqueous extract was stored at 4°C.

Nanoparticle synthesis
We employed the aqueous fruit extract of Annona muricata generated in the preceding stage for the green synthesis of selenium nanoparticles. 10 mL of fruit extract was mixed with 90 mL of 1 mM aqueous selenium solution before heating at 80°C for 3 hours with constant stirring. The change in hue from yellow to dark brown indicated the development of the SeNPs. Green nanoparticles were separated by centrifugation at 15,000 g for 20 minutes. This procedure was performed three times to remove...
the free silver associated with AM-SeNPs. AM-SeNPs were the final green-synthesized silver nanoparticles, which were freeze-dried and stored at 4 °C until further use.

**MIC Test**
The in-vitro antifungal activity of AM-SeNP extract was determined using the agar disc diffusion method. The Candida albicans were purchased from and cultured in Sabouraud’s Dextrose Agar (SDA) medium, then well were created for extract loading. The green synthesis of Annona muricata mediated selenium nanoparticles extract was loaded in well at different concentrations of 25 μl, 50 μl, and 100 μl. The zone of inhibition was measured at different time points 1 - 5 hours. The positive and negative control was used in this study to evaluate the antifungal property.

**RESULTS AND DISCUSSION**
In dentistry, Candida albicans causes several infections, current conventional therapy is limited and multi-drug resistant C. albicans are challenging to treat diseases. The biofilm formation is a key feature of antibiotic resistance in C. albicans species and alteration of the cell membrane, and overexpression of efflux pumps also plays a pivotal role in resistance to antibiotics.(13) Candida species are grown with several pathogenic bacteria and cause dental-related inflammatory infections. Most antibiotics in fungal treatment have generalized adverse effects, paving the path for other approaches of natural therapy (14). In this study, we have synthesized Annona muricata-mediated selenium nanoparticles (AM-SeNP) and assessed their antifungal activity against Candida albicans.

Plants have been utilized as remedies since ancient times. The key benefits of employing plants as alternative medicine include their versatility and adaptability, their availability and cost in the region, and, most importantly, the reduction of unpleasant effects(17). Plants are a great alternative to costly medicines due to their widespread acceptance in low- and middle-income nations, low cost, and low amount of technological input required. As a result, if supported by scientific evidence, plant extracts may show to be better and safer alternatives (18).

Several studies have concluded that Candida albicans is a significant risk factor for precancerous lesions of the oral mucosa, as well as mild and severe dysplasia (in the dysplastic and malignant transformation of oral lesions)(19). Moreover, Candida's ability to generate carcinogens like nitrosamine and acetaldehyde, as well as induce pro-inflammatory cytokines, could be a risk factor for oral cancer development(20). Indeed, the potential function of yeast in neoplastic processes in the oral cavity is still being debated. There is evidence that Candida is a direct cause of oral cancer only when the infection is chronic, deep, and accompanied by risk factors like tobacco and alcohol usage (21).

Almost the majority of the natural extracts had antifungal activity against C. albicans (both in vivo and in vitro), notably A. muricata, Lawsonia inermis, Pelargonium graveolens, Camellia sinensis, Mentha piperita, and Citrus latifolia are possessing the greatest antifungal activity. The main antifungal component were phenolic substances such as gallic acid, thymol, and flavonoids (particularly catechin), polyphenols such as tannins, terpenoids, and saponins. The addition of nanotechnology improves the antifungal effects of these natural chemicals significantly (22).

During our 12-hour experiment, A. muricata aqueous fruit extract successfully produced AM-SeNPs from sodium selenite. The development of brick-red color was used to confirm the synthesis of SeNPs. In this study MIC test revealed that 100ul of green synthesized AM-SeNP significantly inhibits C. albicans growth when compared to the standard antibiotics. We have observed the zone of inhibition and OD value at a different time points, at 5hrs incubation the 100 ul of green synthesized AM-SeNP inhibits the 75% of C. albicans growth when compared to the positive control, while standard antibiotics are inhibits 65% of C. albicans growth (Figure 1 and Figure 2). At each time point from 1 hour to 5 hours, the green synthesized AM-SeNP significantly increases the inhibition of C. albicans growth (Table 1).

The A. muricata plant extract has strong antimicrobial activity, that effectively inhibits the growth of several bacteria Escherichia coli, Staphylococcus aureus, Vibrio cholera, and Salmonella spp. A. muricata were also used.
Green synthesis of Annona muricata mediated selenium nanoparticles and its antifungal activity against Candida albicans

to treat various diseases including cancer, diabetes, diarrhea, dysentery, fevers, hypertension, sores, internal ulcers, and liver disorders(23) A. muricata have various bioactive compounds including acetogenins, that possess anti-tumour, anti-viral and anti-microbial property. Furthermore, the plant extracts consist of various bioactive components, including atherosperminine, asimilobine, annomuricin, annopentocin, annohexocin, loliolide, caffeic acid, normuciferine rutin, xi-anomuricine, kaempferol-3O-rutinoside, s-norcorydine, xylopine, and sucrose (24).

Previous research found that aqueous leaf extract of A. muricata has strong antimicrobial activity against Streptococcus mutans, Pseudomonas aeruginosa, Staphylococcus aureus, and C. albicans. That effectiveness was similar to gold standard controls such as Ciprofloxacain/Fluconazole and Chlorhexidine 0.2% (30). The A. muricata seeds are rich in various flavonoids, including terpenoids, and acetogenins which also have significant anti-fungal activity against C. albicans (25). Another study similarly founds that ethanolic extract of A. muricata significantly reduces the C. albicans growth and cell density. The extracts affect the fungal plasma membrane and disrupt the cell wall integrity. Interestingly a study by Rosaiah discovered that AM-SeNPs have significant anti-microbial and anti-oxidant properties (23). Therefore AM-SeNPs could be a promising treatment approach for treating various infectious diseases and non-infectious diseases including cardiovascular disease and cancers.

FIGURE 1: Result of AM-SeNPs at different concentrations 25, 50, and 100 ul with Positive control and Standard drug in SDA plates after 5 hours of incubation.

FIGURE 2: The Graph represents the optical density value of C. albicans growth in different concentrations of AM-SeNPs, Positive control, and Standard. The 100ul of SeNPs significantly inhibits the C. albicans growth which is greater than the standard drug.
TABLE 1: The OD value of C. albicans growth in different drug concentrations and time points.

<table>
<thead>
<tr>
<th>Drugs / Time point</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>4 h</th>
<th>5 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 μL</td>
<td>0.551</td>
<td>0.531</td>
<td>0.512</td>
<td>0.339</td>
<td>0.279</td>
</tr>
<tr>
<td>50 μL</td>
<td>0.511</td>
<td>0.47</td>
<td>0.429</td>
<td>0.276</td>
<td>0.237</td>
</tr>
<tr>
<td>100 μL</td>
<td>0.482</td>
<td>0.428</td>
<td>0.383</td>
<td>0.259</td>
<td>0.179</td>
</tr>
<tr>
<td>Positive Control</td>
<td>0.588</td>
<td>0.619</td>
<td>0.657</td>
<td>0.695</td>
<td>0.743</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>0.503</td>
<td>0.476</td>
<td>0.391</td>
<td>0.315</td>
<td>0.267</td>
</tr>
</tbody>
</table>

CONCLUSION
The green synthesizes nanoparticles are a promising treatment strategy to control antimicrobial-resistant pathogens. In this study our green synthesized Annona muricata-mediated selenium nanoparticles (AM-SeNP) significantly inhibit the C. albicans growth at 100μl concentration in vitro, their efficacy is greater than standard antibiotics. It may use to treat various oral diseases including oral cancer due to its antitumor activity. Further studies in an animal model, human, and characterization of A. muricata plant composition may help to identify the potential role in antimicrobial therapy.

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