Original Article

Evaluation of antifungal activity of vanilla pods silver nanoparticles against various oral candidal species: An *in-vitro* study

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Abstract

Background: The oral health of individuals is undoubtedly affected by the fact that fungal microorganisms are currently resistant to the conventional antifungal drugs used. Still, there are numerous emerging therapeutic alternatives available like phytotherapy. The phytochemistry of various plant species has indicated that phytochemicals could be a better source of medicine with outstanding antifungal, healing, anti-inflammatory spectrum, and antioxidant properties.

Aim: To evaluate the antifungal efficacy of silver nanoparticles of vanilla pods against *Candida albicans*, *Candida glabrata*, and *Candida krusei* and to compare with Fluconazole.

Materials and Methods: The vanilla pods were procured from the organic store. The aqueous extracts were prepared. Ten percent of the vanilla extract was utilized to generate the silver nanoparticles. The generated silver nanoparticles were characterized visually based on color change and confirmed by UV spectroscopy. Disk diffusion method was used to determine the antifungal activity of the vanilla pods reinforced silver nanoparticles at different concentrations (20 µl, 40 µ, 40n µ, and 80 µand 80ratio*C. albicans*, *C. glabrata,* and *C. krusei*. The antifungal efficacy of the produced nanoparticles was evaluated against a standard of ketoconazole (30 µ30.

Results: Vanillin silver nanoparticles exhibit commendable antifungal activity against *C. albicans*, *C. glabrata* which is equivalent to that of ketoconazole, and amplified activity against drug-resistant *C. krusei* was evident.

Conclusion: Silver nanoparticles from vanilla pods were studied and their wider zones of inhibition indicated exemplary antifungal efficacy compared with conventional antifungal drugs like ketoconazole.

Clinical significance: Future research on vanillin nanocarrier systems might enhance their stability, bioavailability, and bioactivity. As an outcome, with some promising advances in this area, it would be captivating to pursue a systematic investigation into the potential effects of vanillin at the cellular and molecular levels. This will enable us to further assess its applicability as an active biopharmaceutical ingredient in mouthwashes, probiotic lozenges, and denture resin for use as an effective strategy against the biofilm growth of various Candida species.

Keywords: Antifungal efficacy, drug resistance, phytotherapy, vanillin silver nanoparticles

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INTRODUCTION

Oral microflora or microbiome represents the intricately interconnected communities of a diverse group of microorganisms, primarily bacterial and fungal species, protozoans, and occasionally viruses that reside in the oral cavity which harbors the second largest microbial community next to the gut.[1] The multifaceted harmony between resident species in the oral cavity is responsible for maintaining an optimal state (in symbiosis) or a disease-associated state (in dysbiosis). Dysbiosis in oral homeostasis caused by the host's weakened immune response owing to age, diabetes, stress, tobacco use, or increased virulence facilitates the transformation of this commensal organism into pathogenic microbes.[2] Among these, fungal infections have a profound impact on health resulting in the main cause of morbidity and mortality globally. Pathogenic fungal species include *Aspergillus fumigatus*, *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Blastomyces dermatitidis*, Zygomycetes class, *Coccidioides immitis*, *Paracoccidioides brasiliensis*, and Candidal species.[3]

The spectrum of fungal infection ranges from superficial skin or mucosal involvement to invasive disease, which has become an important healthcare-associated bloodstream infection that contributes to increased morbidity and mortality. An increase in invasive fungal infections is paralleled by an increasing incidence of diabetes, an increase in immunosuppressive therapies (e.g. dialysis, transplantation, central venous catheters, parenteral nutrition, and broad-spectrum antibiotic use), and the advent of the human immunodeficiency virus.[4] Candidiasis is the most common fungal infection caused by causative *C. albicans* predominantly along with *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. guillermondii*, *C. krusei*, *C. Kiefer*, and *C. dubliniensis*. *C. albicans* is a leading cause of nosocomial infections, with mortality rates often exceeding ~40% despite treatment. Non-albicans Candida (NAC) species are also of concern, with drug-resistant isolates on the rise.[5]

The fact that there are only three classes of antifungal medications available to treat systemic fungal infections increases the impact on human health [Figure 1]. The azoles that target ergosterol biosynthesis, the echinocandins that prevent the synthesis of fungal cell walls, and the polyenes that bind to ergosterol in the fungal cell membrane and cause cell lysis are some examples of these.[6] Since ancient times, humans have sought cures for diseases in nature, as evidenced by phytotherapy, a traditional practice of treating health problems with medicines derived from plants or herbs. Because of its antimicrobial, antiplaque, analgesic, antioxidant, and anti-inflammatory properties,

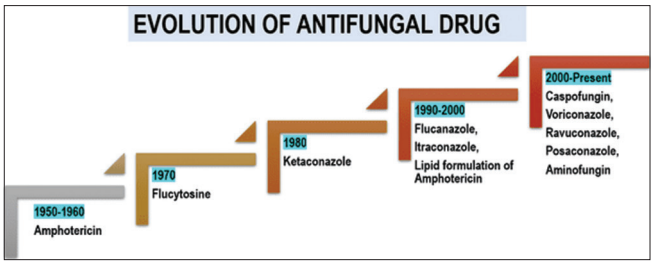

Figure 1: Evolution of Various Antifungal Drugs

lower toxicity and side effects, low price, and good therapeutic potential, this can be an emerging alternative to conventional medications available.[7] Phytotherapy can also be employed as a replacement for current treatments for oral health issues. Multiple research studies have demonstrated that these natural herbal treatments have fewer side effects and are safer than conventional treatments.[8]

Traditional medicinal practices are utilized by 80% of people in developing countries to fulfill and/or enhance their basic health needs as reported by the World Health of Organization (WHO). Nanotechnology techniques have been extensively researched in the medical field in recent years. Breakthroughs in nanotechnology science can potentially reshape the emergence of formulations based on natural products.[9] The combination of nanotechnology with the development of a drug delivery system based on nanoparticles and bioactive natural compounds is very appealing and has rapidly grown in recent years and could be a viable alternative to developing more recent pharmaceutical formulations that effectively battle fungal infections and overcome fungal multi-resistance to existing drugs.[10,11]

The vanilla bean, obtained from the Orchidaceae family members Vanilla planifolia and Vanilla tahitensis, is the source of vanilla extract, one of the most desired and widely used food flavorings worldwide. Vanilla is employed as the most preferred flavor and fragrance ingredient in ice creams, confectioneries, the dairy industry, perfumes, and pharmaceuticals, but it also has complementary medical applications.[12] Literature review on the therapeutic application of vanillin revealed few studies which found that it possesses a spectrum of properties ranging from anti-inflammatory, anti-cancerous, antimicrobial, and antioxidant to neuroprotective, nephroprotective, wound healing, and preventing glycation of insulin.[13] The present study was drafted on this background to effectively evaluate the antifungal efficacy of silver nanoparticles mediated vanilla pods on *C.albicans*, *C.glabrata*, and *C. krusei* to formulate herbal-based drugs to combat the multi-fungal

drug resistance, enhanced bioavailability, minimal side effects, and site-specific targeted approach.

MATERIALS AND METHODS

Aqueous herbal extract preparation

Vanilla pods were obtained from the local organic store [Figure 2]. Ten grams of the sample were weighed and soaked in 100 ml of distilled water, macerated, and incubated overnight at room temperature [Figures 3 and 4]. Following the incubation, the extract was filtered using sterile filter paper. The resultant filtrates of the vanilla pod were used for the synthesis of silver nanoparticles.

Synthesis of silver nanoparticles

Ten milliliters of the aqueous extract of the sample was added to 90 ml of 1 mM, 3 mM, and 6 mM aqueous AgNO3 solution and kept for 15–20 mins at room temperature, respectively. This aqueous extract acts as a reducing and stabilizing agent for the AgNO3 solution. The change in color of the solution from pale yellow to reddish brown and black indicated the reduction of Ag^{+} ions to Ag^{0}. The formation of reddish brown and ruby red solution established the preliminary confirmation for the formation of plant-extract-mediated synthesis of silver nanoparticles [Figures 5 and 6].

Characterization of silver nanoparticles

The synthesized AgNPs from vanilla pods were further characterized by UV visual spectroscopy. The spectral response of synthesized AgNPs was monitored by UVized by UV spectrophotometer in the wavelength range of 200–800 nm (Thermo Scientificwavelength 201). Double distilled water was used as a blank control [Figure 7].

Antifungal activity analysis

The Candidal species were inoculated on HiCrome Candida differential agar (HiMedia laboratories) and incubated at 37°C for 24–48 hours, and the species were identified by the type and color of the colonies on the chromogenic

Figure 2: Vanilla pods

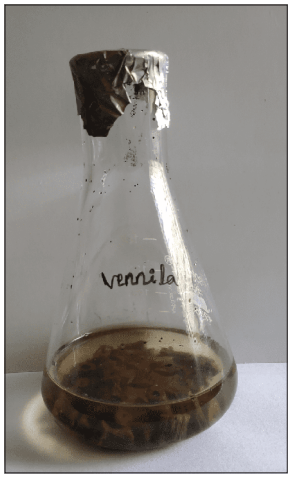

Figure 3: Ethanolic extract of vanilla silver pods

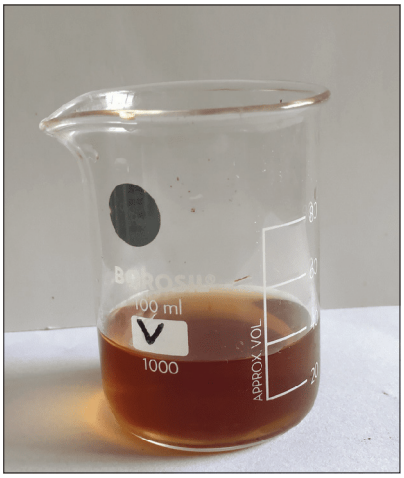

Figure 4: Overnight incubation of vanilla extract

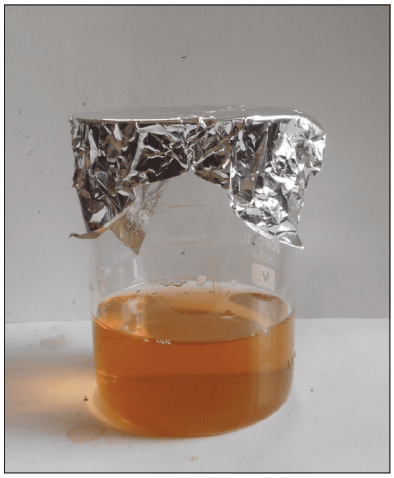

Figure 5: Preparation of silver nanoparticles of vanilla

medium. The colony morphology was exhibited by light green-colored smooth colonies of *C. albicans*, cream to white smooth colonies of *C. glabrata*, and purple fuzzy colonies of *C. krusei* [Figure 8].

The antifungal activity of the sample was determined by the agar disk diffusion method on the Potato Dextrose agar (PDA) medium. The fungal inoculums were spread on the solid culture plates with sterile swabs moistened with the fungal suspension. Wells were made using a cork borer. The samples (silver nanoparticles of green coffee and green tea) and positive control (ketoconazole 30 µ0toconazole terile swabs moistened with the fungal suspensiothen incubated for 24 to 48 hours at room temperature. Finally, the efficacy of the antifungal activity of each sample at different concentrations (20 µ20 40 µ00 60 µ00 and 80 µ0d was determined by measuring the diameter of the zone of inhibition.

RESULTS

The green synthesis of silver nanoparticles with the help of a vanilla pod was carried out. On mixing the aqueous plant extract of vanilla pods in a 3 mM solution of AgNO3 solution reductive color change from pale yellow to dark brown was seen. UV visual spectroscopy of the silver nanoparticles of vanilla pods revealed a specific adsorption band at 455 nm. This peak was attained after a 2-hour reaction period and the broad absorption band that was seen indicated that the distribution of the nanoparticles was polydisperse.

The antifungal activity of the AgNPs of vanilla was evaluated against *C. albicans*, *C. glabrata*, and *C. krusei* via the disk diffusion method. The effect of different concentrations of Vanillin AgNPs against all three species was reported [Table 1]. Antifungal activity of the vanilla pod was started at the initial concentration of 20 able 1] t concentrations ofand at 455 nm. ThisO advanced. The zone of inhibition was higher for *C. Krusei* compared to that of the standard used, that is, ketoconazole. Vanillin silver nanoparticles exhibit strong antifungal activity against the Candidal species with a zone of inhibition as 20 mm, 25 mm, and 22 mm for *C. albicans, C. glabrata*, and *C. Krusei*, respectively, which was equivalent to that of ketoconazole [Figures 9-11].

DISCUSSION

Fungi cause an extensive spectrum of human diseases, from allergic syndromes to superficial, disfiguring, and potentially fatal invasive fungal diseases (IFDs), affecting

Figure 6: Overnight incubation of vanilla silver nanopartciles

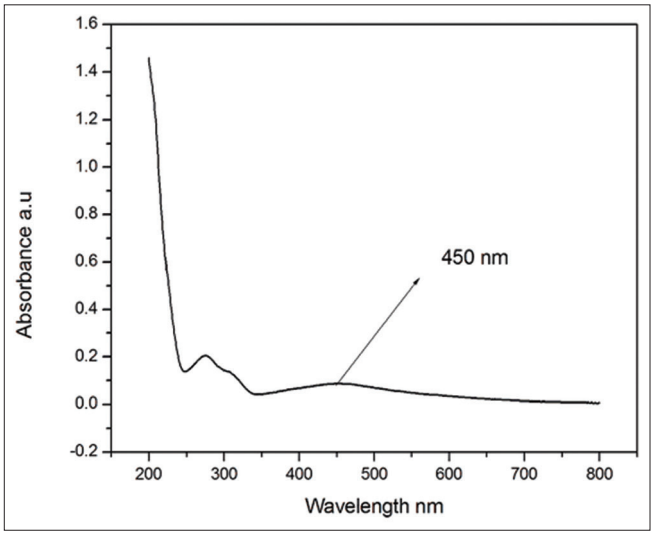

Figure 7: UV spectrophotometer analysis

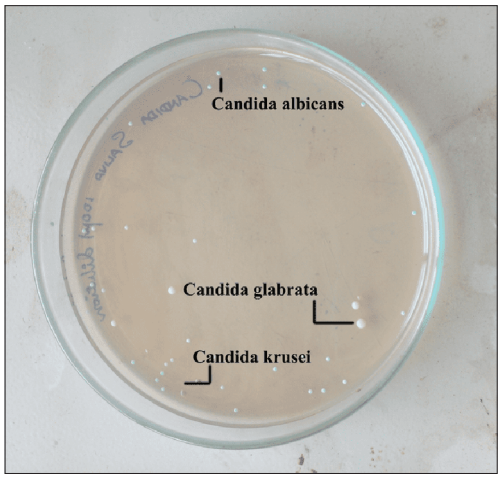

Figure 8: Light green-colored smooth colonies of *candida albicans*, cream to white smooth colonies of *candida glabrata*, and purple fuzzy colonies of *candida krusei* on hichrome candida differential agar

over a billion people worldwide. Polyenes, azoles, echinocandins, and the pyrimidine analog 5-flucytosine

are the four classes of systemically acting antifungal drugs currently in use with drug resistance due to a combination of underlying host immune deficiencies, antifungal drug properties (pharmacokinetics, pharmacodynamics, and drug-drug interactions), and fungal characteristics such as diverse cell morphologies and antifungal tolerance is evident.[14] The alarming rise in the public health burden has been officially acknowledged with the incorporation of pathogens, namely, *Aspergillus fumigatus* and *C. auris* on the United State Center for Disease Control (CDC)'s urgent antimicrobial resistance (AMR) threat list published in 2019.[15]

Although Candida is the normal commensal of the oral microbiota, an imbalance in homeostasis will end up in the most common opportunistic infection, Candidiasis. Apart from *C. albicans*, other Candidal species that are known to cause disease include *C. glabrata*, *C. krusei*, *C. tropicalis*, and *C. auris*. Of these, *C. auris* and *C. krusei* are the emerging drug-resistant species as stated by CDC.[16] Bioactive components extracted from natural products are regarded as goldmines for the discovery of novel drugs. Vanillin besides being the most promising flavoring agent also has antifungal properties established by few studies.[17] The mechanism of antifungal resistance exerted by vanillin is in two ways: [Figure 12]

(i) Inhibition of growth, filamentation, and biofilm development and
(ii) Preventing the conversion of yeast to invasive hyphal forms.

Extracts from various parts of this orchid, such as leaves and beans, have been shown to possess alkaloid substances

Table 1: Zone of Inhibition of Vanillin Extract at Various Concentrations

Candidal species	Zone of inhibition in mm				
	Vanilla silver nanoparticle extract				Fluconazole (30 µl)
	10 µ	20 µ	40 µ	60 µ	
C. albicans	10 mm	13 mm	17 mm	20 mm	21 mm
C. glabrata	20 mm	21 mm	23 mm	25 mm	25 mm
C. krusei	15 mm	18 mm	20 mm	22 mm	15 mm

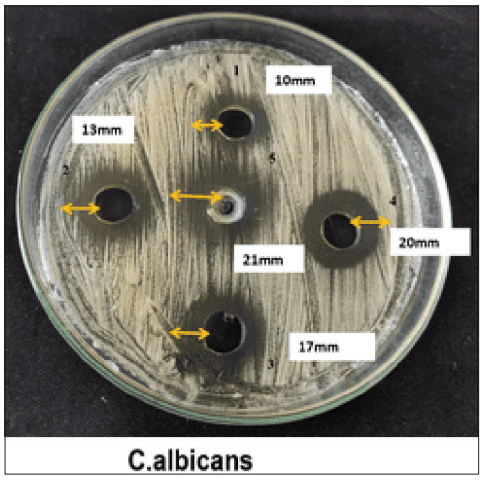

Figure 9: Antifungal activity of *candida albicans*

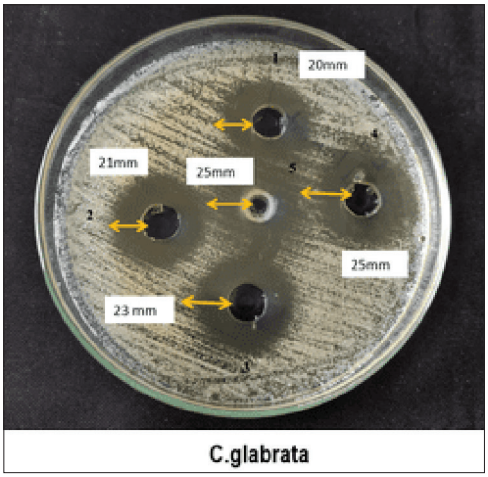

Figure 10: Antifungal activity of *candida glabrata*

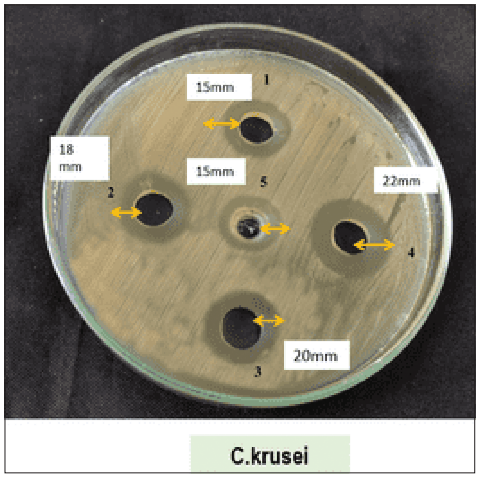

Figure 11: Antifungal activity of *candida krusei*

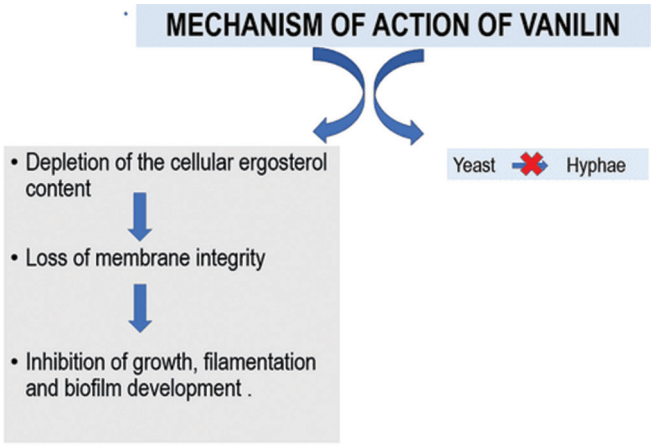

Figure 12: Mechanism of action of vanillin by two possible ways

and phyto-compounds that are potent anti- fungal. However, an extensive literature search revealed few clinical trials or experiments done using vanilla pods or vanilla leaves. Therefore, the present study aimed to explore the antifungal properties of the newly prepared vanilla planifolia extract obtained from vanilla pods. To the best of our knowledge, this study was the first of its kind to evaluate the antifungal efficacy of vanilla pods against sub-species of Candida, namely, *C. albicans*, *C. glabrata*, and *C. krusei*.

Vanillin silver nanoparticles used in our study exhibit strong antifungal activity against the Candidal species with a zone of inhibition of 20 mm, 25 mm, and 22 mm for *C. albicans*, *C. glabrata*, and *C. Krusei*, respectively, whereas the control used in our study (ketaconazole) exhibits 21 mm, 25 mm, and 15 mm indicating the maximal antifungal potency of the vanillin. The antifungal effectiveness of vanillin against multidrug resistance *C. krusei* is another important finding from our investigation. *C. krusei* exhibits a praiseworthy result that is better than the control employed in our investigation. Our work proves to be a breakthrough in it, as previous herbal investigations done on this species demonstrated no or minimal antifungal effect against this organism.[18]

Raut JS *et al.* in 2013 found the significant ($P < 0.05$) reduction in biofilm development of *C. albicans* was observed in the presence of 500 μg ml–1 vanillin.

Concentration-dependent decrease of cellular ergosterol in the presence of vanillin indicated that inhibition of ergosterol biosynthesis may be a probable target.[19] This was supported by our study wherein *C. albicans* exhibits a good zone of inhibition which is equivalent to that of ketoconazole.

Kim JH *et al.* in 2014 inferred that RNA-seq of *o*-vanillin and *o*-ethyl vanillin treated *C. neoformans* showed that they caused mitochondrial dysfunction and triggered oxidative stress, significantly reducing their growth and explaining another mechanism by which vanillin exhibits its antifungal efficacy.[20] Romero-Cortes *et al.* in 2019 inferred that vanillin (250 mg/L) decreased the growth of *Alternaria* strains, suggesting its fungistatic behavior. The lag time of the fungal life cycle was increased from an initial 50 h to 112 h and also inhibition of mycelial growth of up to 37.5% was observed.[21]

Kamath *et al.* 2022 used leaf extracts of vanilla and concluded that no antifungal activity against *C. albicans* which is contrary to our results. However antibacterial activity against *Staphylococcus aureus, Streptococcus mutans, and Enterococcus* was found.[22] This disparity could be because of the usage of vanilla pod extract in our study along with the application of silver nanoparticles-aided vanillin extract, enhancing the phytochemicals delivery.

In this study, the efficacy of vanilla pods silver nanoparticles was assessed by measuring the zones of inhibition exhibited by the nanoparticles at different concentrations via the disk diffusion method. The results obtained against the three species are commendable which makes them a promising alternative to conventional antifungal drugs with further validation obtained from the future studies.

Clinical significance

Future research on vanillin nanocarrier systems might enhance their stability, bioavailability, and bioactivity. As an outcome, with some promising advances in this area, it would be captivating to pursue a systematic investigation into the potential effects of vanillin at the cellular and molecular levels. This will enable us to further assess its applicability as an active biopharmaceutical ingredient in mouthwashes, probiotic lozenges, and denture resin for use as an effective strategy against the biofilm growth of various Candida species.

CONCLUSION

The study proved the silver nanoparticles were synthesized from vanilla extract successfully. The synthesized AgNPs showed excellent antifungal activity against all three species involved in our study. Antifungal activity against *C. krusei* showed exemplary results, which was not obtained from previous herbal studies. With these promising results, further studies using the vanilla extract will explore the further potential and application of this extract into dentistry by incorporating this silver nanoparticle mediated vanillin extract into mouthwash, lozenges, and in denture thus minimizing the complication and promoting the quality of life of the patients.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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