

Research Article

The Disinfectant Effect of Modified Hydrothermal Nanotitania Extract on *Candida albicans*

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Candida albicans is a pathogenic yeast typically associated with diseases such as HIV, AIDS, and other immunocompromised conditions. It is rarely the cause of fatalities and is commonly treated with drugs administered orally or intravenously. In this experiment, *Candida albicans* was tested with an extract of modified hydrothermal nanotitania to identify whether the material is capable of inhibiting the organism's growth. The extract was mixed with the *Candida albicans* in a culture media preparation to determine whether the organisms were able to grow. The test showed that modified hydrothermal nanotitania inhibits the growth of this organism.

1. Introduction

A fungus is a common single-celled or eukaryotic organism. In the fungal community, the most commonly found dimorphic type is *Candida albicans*. It frequently causes various local medical infections of the nails, vagina (vaginitis), and respiratory system. Candida infection or candidosis rarely occurs spontaneously as it needs favourable conditions for growth. Those patients most prone to developing candidosis are those receiving broad-spectrum antibiotics and those with diabetes mellitus. In these groups, candida develops in the saliva [1]. Other factors that can lead to candidosis are the suppression of the immune system through, for example, immunodeficiency viral infection, and the loss of skin integrity, as in the case of eczema. In certain instances, candidosis may be preceded by a fever of unknown origin leading to systemic fungal infection [2].

Treatment of candidosis is via local ointment or oral antifungal depending on the type of infection. Drugs such as amphotericin B and caspofungin are commonly used to treat the condition [3]. Certain antibiotics, such as rapamycin, are also used [4].

Unfortunately, candida can sometimes be resistant to antifungal treatment. For example, candidosis in the oropharynx has been reported as being resistant to fluconazole [5]. It is speculated that this drug resistance is due to genetics [6, 7]. In another study, candida was found to have the ability to form a biofilm which may contribute to antifungal resistance [8]. This biofilm could in fact be a multifactorial mechanism according to research conducted using an efflux pump [9]. Another hypothesis relating to biofilm resistance is that the changes in sterol composition with altered membrane permeability transform antifungals into candida [10].

TABLE 1: Monitoring of *Candida albicans* growth on tested agar at 24-, 48-, and 72-hour intervals.

Test/hours of incubation	24 hours	48 hours	72 hours
Candida only (negative control)	Growth	Growth	Growth
Undoping (positive control) + candida	No growth	No growth	No growth
TiO ₂ with 0.01% silver + candida	No growth	No growth	No growth
TiO ₂ with 0.03% silver + candida	No growth	No growth	No growth
TiO ₂ with 0.05% silver + candida	No growth	No growth	No growth

The surface roughness of titanium dental implants has been associated with the significant resistant properties of *Candida albicans* [11]. In an experiment, *Streptococcus mutans* was combined with *Candida albicans* biofilm, and corrosion of the titanium was observed due to pH-lowering [12]. These two organisms—*Streptococcus mutans* and *Candida albicans*—were both able to produce biofilm in vivo and invitro [13]. As with *Streptococcus mutans*, *Candida albicans* also facilitates *Staphylococcus aureus* in systemic infection [14]. The biofilm development on titanium was reduced by covalently binding it with vancomycin and caspofungin [15]. Interestingly, candida did not produce the same results on zirconia ceramic implants [16].

Nanotitanium dioxide (nanotitania) has been successfully extracted via a modified hydrothermal process [17–19]. It is well-known that titanium dioxide has a low energy band gap which enables excited electrons to jump from the valance band to the conduction band and to also easily perform photocatalytic activities. The manipulation of these low band gap activities has been performed in previous studies of dye-sensitized solar cells [20, 21]. Titanium dioxide (TiO₂) is currently being used against bacterial invasion. A reduction in *E. coli* development was precipitated by gradually increasing the concentration of TiO₂ nanoparticles used [22]. In other research, the combination of TiO₂ with silver was found to act as antimicrobial material [23]. Furthermore, naturally produced synthesized TiO₂ nanoparticles extracted from the *Psidium guajava* aqueous leaf are connected with their antibacterial action against *S. aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* [24].

The mechanism utilised by titanium dioxide to stop bacterial activity has been the subject of several experiments. Nanoparticles of TiO₂ have been shown to inhibit the growth of the bacteria *Streptococcus mutans* orally through the suppression of the 24-hour lactate production mechanism [25]. In addition, TiO₂-based nanocomposite when used for its photocatalytic properties reduces the expression of specific genes and proteins in *P. aeruginosa* PAO1 cells [26].

In this study, *Candida albicans* was combined with a nanotitania extract, which had been modified hydrothermally to detect whether nanotitania has the ability to reduce and/or eliminate organisms. During the experiment, the modified hydrothermal nanotitania extract was mixed together with the organisms in culture media to determine the organism's growth potential.

This study is expected to show similar results as those obtained from our previous test of modified hydrothermal nanotitania extract which revealed inhibition of *Staphylo-*

coccus aureus and *E. coli* growth [27, 28]. Previous experiments with nanotitanium dioxide have also resulted in suppressing its mutagenic capability [29] and also its toxicological properties [30].

The findings of this study are that nanotitania extract when modified hydrothermally and combined with nanoparticles and a high percentage of silver is able to resist the growth of *Candida albicans*.

2. Materials and Methods

Hydrothermally modified nanotitania extract was mixed with multiple concentrations of silver (0.01%, 0.03%, and 0.05%) in a test plate. To test these materials, a control of titanium dioxide was used without any silver mixing (undoped). One of the key aspects of the nanotitania to note is it was not diluted in water, dimethyl sulfoxide (DMSO), or sulphuric acid.

A sample of *Candida albicans* was obtained from a hospitalized patient at Hospital Universiti Sains Malaysia which was then grown in the microbiology lab of the School of Medical Sciences, Universiti Sains Malaysia.

The test was done to monitor whether the nanotitania extract was able to prevent the growth of the bacteria and fungus by mixing it with the agar media before the addition of the bacteria/fungus (the method used by Ahmad et al., 2013 [17]). In addition, a control media without the nanotitania extract was used in which the candida was allowed to grow.

2.1. Preparation of TiO₂. Suspensions of 1g/ml TiO₂ (together with 0.01%, 0.03%, and 0.05% silver) and undoped (plus a positive control containing TiO₂ and no silver) were prepared by mixing 1g of TiO₂ in 1 ml of Mueller Hinton Broth (MH) before the addition of 1 ml *E. coli* equivalent 0.5 MacFaland. Agar containing only bacteria without TiO₂ was used as a negative control. The suspensions were then placed in an incubator with a shaker for 24 hours at a temperature of 37°C. A 100 µl suspension was inoculated on MH agar, and the bacterial inoculation was spread with a hockey stick. The agar plates were observed after 24, 48, and 72 hour incubation periods to detect any bacterial growth.

Candida albicans was prepared on an agar plate (using the method mentioned above in (a)). After 24 hours of incubation, the agar plate containing the bacteria was checked for evidence of growth. The TiO₂ containing 0.01%, 0.03%, and 0.05% silver and undoped (containing TiO₂ only) were weighed on the analytical balance digital precision electronic scale for 1 gram each. The substances then were added and distributed on the prepared agar plate containing the growth

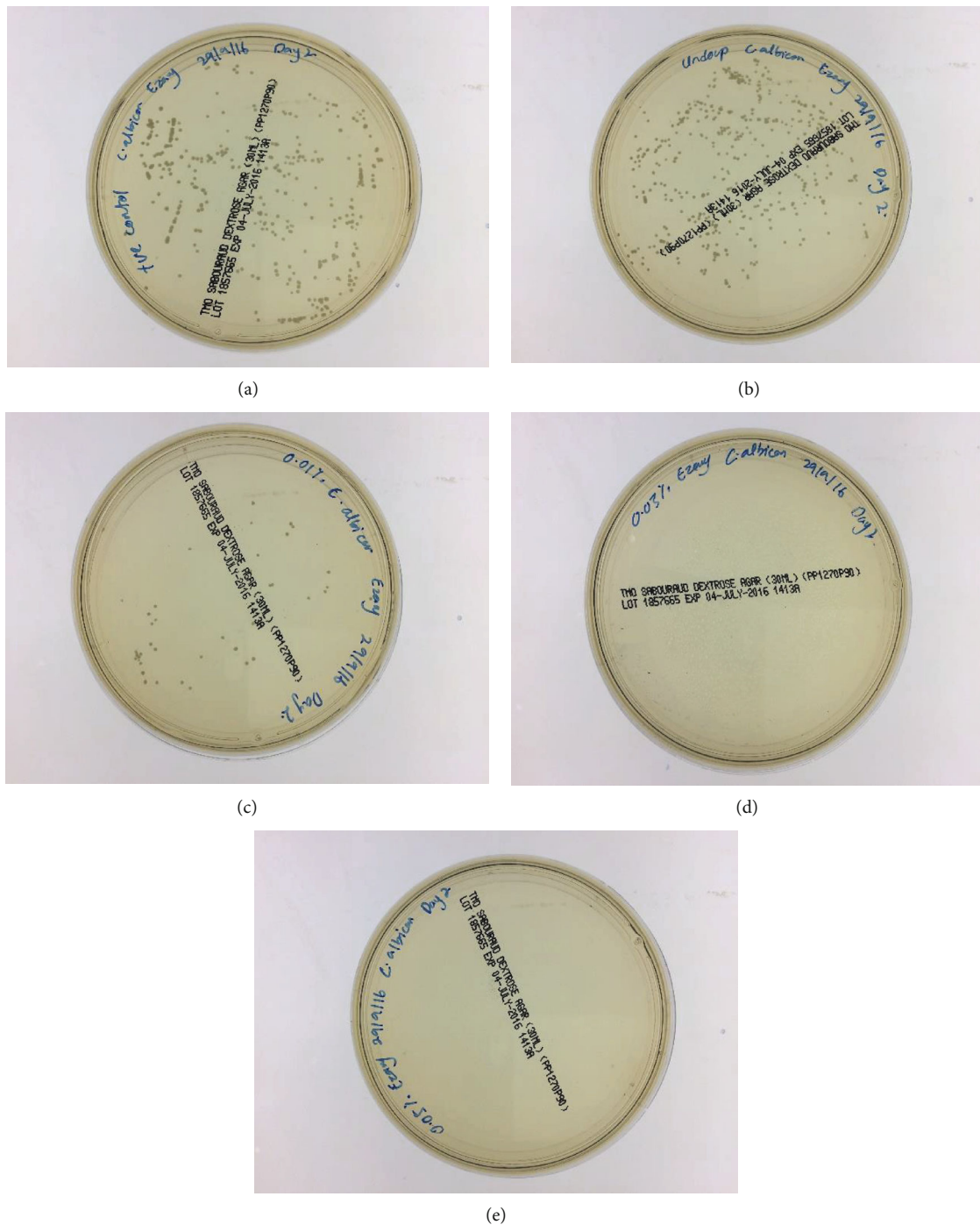


FIGURE 1: Testing modified hydrothermal nanotitania extract with *Candida albicans*: (a) control media-containing candida only; (b) TiO_2 only; (c) TiO_2 containing 0.01% silver with candida; (d) TiO_2 containing 0.03% silver with candida; (e) TiO_2 containing 0.05% silver with candida. Note: no *Candida albicans* growth was detected in the agar plates (d) and (e). However, agar plate (c) contained a significantly smaller candida colony.

bacteria (one substance on one plate containing the bacteria) under the fume hood (room temperature). A plate containing the bacteria was used as a control under the same conditions. The plates were each observed for changes in morphology. Specifically, changes in shape, size, colour, plus any other differences were noted and times recorded with the aid of a stopwatch.

3. Results and Discussion

Modified hydrothermal nanotitania extract containing titanium dioxide (TiO_2) mixed with 0.01%, 0.03%, and 0.05% silver nanoparticles and "titanium dioxide only" is more capable of stopping the growth of *Candida albicans* than the control media.

Titanium dioxide (TiO₂) comprising silver concentrations at various percentages (0.01%, 0.03%, and 0.05%) and TiO₂ only are able to inhibit the growth of *Candida albicans* on an agar plate in comparison to a control containing no nanoparticles. In various agars containing the nanotitania extract consisting of modified hydrothermal nanotitania extract mixed with silver (at 0.01%, 0.03%, and 0.05% concentrations), no *Candida albicans* growth was observed after 24, 48, or 72 hours. In the negative control agar without the nanotitania extract, bacterial growth was noted. The results are shown in Table 1 incorporation with Figure 1.

4. Discussion

The deterrence of *Candida albicans* development in the oral cavity and other parts of the body is vital. Susceptibility to candidosis infection, as often noted in HIV and AIDS patients, has been the catalyst for research into *Candida albicans* prevention.

In this experiment, modified hydrothermal nanotitania extract containing TiO₂ was mixed with differing percentages of silver (0.01%, 0.03%, and 0.05%) together with an undoped sample (containing TiO₂ only) that were tested against *Candida albicans*. The main objective was to investigate the capability of this modified hydrothermal nanotitania extract to prevent the *Candida albicans* growth.

The main problem with the nanotitania extract was its inability to be diluted in water, dimethyl sulfoxide (DMSO), or sulphuric acid. In this experiment, the method used to identify the ability of these substances to prevent the growth of *Candida albicans* was the same one as used by Ahmad et al., 2013 (mentioned above). The results showed that the modified hydrothermal nanotitania extract can retain antifungal properties for up to 72 hours after addition to the agar containing the extract and the organisms. These results explain the potential of these substances to be used for at least 72 hours as antibacterial and antifungal agents.

4.1. Limitations of the Study. The modified hydrothermal nanotitania extract was not dissolved in water. This limited the range of tests possible for antibacterial properties.

5. Conclusions

In our experiment, the modified hydrothermal nanotitania extract was shown to inhibit the growth of *Candida albicans*.

Data Availability

Details are presented within the article in the form of tables and text in results. Other data will be made available upon request.

Conflicts of Interest

The authors declare no conflict of interest.

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