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Evaluation of antimicrobial property of thymoquinone synthesized with black cumin hydroxyapatite crystals against dental pathogens - An Invitro study

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ABSTRACT

Introduction: Nigella sativa, also known as black seed, black cumin, and/or Habbatul Barakah, is a medicinal herbal plant. Thymoquinone (TQ), one of Nigella sativa's active ingredients, has been shown to be anticarcinogenic, anti-inflammatory, analgesic, and antimicrobial. Hydroxyapatite (HA) is an inorganic mineral with the apatite lattice structure (A10(BO4)6C2). The aim of the current study is to incorporate thymoquinone with blackcumin hydroxyapatite crystals to form a new nanoformulation and to find out its cytotoxicity and antimicrobial properties against dental pathogens.

Materials and method: Streptococcus mutans, Staphylococcus aureus, Enterococcus faecalis, and Candida albicans were the species used. The antimicrobial efficacy of the nanoparticles was evaluated using the agar well diffusion method at different concentrations ranging from 25 micron litres to 100 micron litres. UV double beam spectrophotometers in the wavelength range of 250-750nm were used to characterise the synthesised nanoparticles.

Results: The zone of inhibition against Staphylococcus mutans and Candida albicans was found to be highest at 100 L concentration. As the concentrations of new nano formulation solution increased, the mean zone of inhibition grew.

Conclusion: The study showed that there is potential antimicrobial property of the new nano formulation against Streptococcus mutans and Staphylococcus aureus also there is antifungal activity against Candida albicans

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INTRODUCTION

The oral cavity of humans is home to about 700 bacterial species that coexist peacefully. A disease emerges when this equilibrium is disrupted and the balance changes toward pathogenic organisms (1). Hard tissues such as enamel, dentin, and cementum make up a human tooth. Enamel is the toughest of all tissues, and it protects the tooth's anatomical crown. It's a highly mineralized hard tissue with a 4 percent organic content and a 96 percent inorganic content. High inorganic content makes the enamel as the hardest tissue in the human body(2). Enamel once lost cannot be regenerated or repaired. The most prevalent disease in the oral cavity is the dental caries and is caused by destruction of tooth structure by acids produced from cariogenic bacteria (3,4). Streptococcus mutans are gram positive bacteria found in biofilms attached to surfaces of the tooth structure and is considered as the major cariogenic species as it produces large amounts of acids, thus surpassing non cariogenic organisms and causing demineralization of hard tissues (5).

Nigella sativa, also known as black seed, black cumin, and/or Habbatul Barakah, is a medicinal herbal plant(6,7). It is widely used by millions of Muslims around the world and is associated with religious significance. Nigella sativa seeds have historically been used to treat body aches, nasal inflammation, and intestinal worms. These seeds are thought to be an aphrodisiac which is an active source of ions such as calcium that could aid in the remineralization of tooth structure(8)

Thymoquinone (TQ), one of Nigella sativa's active ingredients, has been shown to be anticarcinogenic, anti-inflammatory, analgesic, and antimicrobial (9–11). In a previous animal study, Shaker and Al-Wafi tested TQ for the prevention of gingival inflammation in a rat model and found promising results (12). In comparison to the control groups, gingival inflammation and periodontal indices were significantly lower in rats treated with TQ. TQ's role in the healing of oral ulcers has also been recorded in the literature (13). In a previous study, Al-Thobity et al found that adding TQ to an acrylic denture base may help prevent Candida albicans from adhering to it (14). As a result, denture stomatitis is avoided. It has been documented that thymoquinone induces an antioxidant response by scavenging various free radicals, with its scavenging power equaling that of superoxide dismutase against superoxide anions.

Thymoquinone can also have anti-inflammatory effects due to membrane lipid peroxidation and eicosanoids (15)(16). Hydroxyapatite (HA) is an inorganic mineral with the apatite lattice structure (A10(BO4)6C2), where Ca, PO4, and OH define A, B, and C, respectively . The Ca/P mole ratio in pure HA is 1.67 since it contains 39.68 percent calcium and 18 percent phosphorus by weight. There are industrial HA goods with Ca/P ratios greater than or less than 1.67. The Ca/P ratio varies, indicating a phase transition between tricalcium phosphate (TCP) and calcium oxide (CaO). CaO is more abundant in HA with a Ca/P ratio greater than 1.67, and vice versa. HA crystals in the form of plates or needles are 40 to 60 nanometers long, 20 nanometers wide, and 1.5 to 5 nanometers thick. The arrangement of various HA crystalline sizes and shapes supports the structural stability, hardness, and function of this tissue (17).

The aim of the current study is to incorporate thymoquinone with blackcumin hydroxyapatite crystals to form a new nanoformulation and to find out its cytotoxicity and antimicrobial properties against dental pathogens.

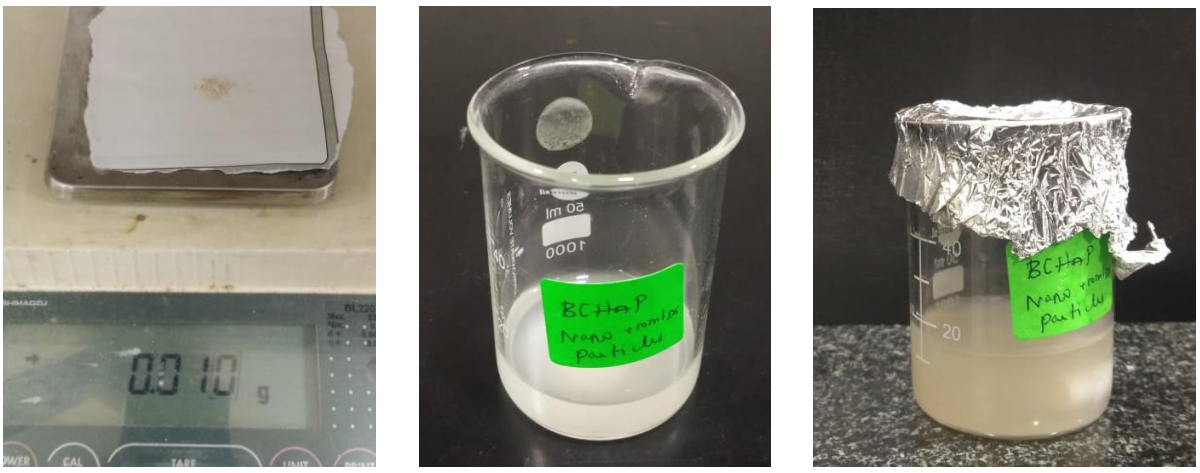
MATERIALS AND METHODS

Study design: In vitro study

Study setting: The study was done in BLUE LAB (microbiology and biochemistry lab), saveetha institute of medical and technical science (SIMATS), Chennai.

Ethical approval: Ethical approval was obtained from institutional review board, Saveetha University (SIMATS).

Green synthesis process: 0.010 g of black cumin hydroxyapatite powder (figure 1a) which was prepared for the previous study was mixed in 10mL of distilled water (figure 1b). Thymoquinone was brought from sudhakar biologicals and chemicals, Chennai. 0.50 g of thymoquinone was weighed and mixed in 100mL of distilled water and heated in the heating mantle for 5 mins at 50 degree celsius. Then 10mL of already mixed black cumin hydroxyapatite crystal was mixed in 10mL of thymoquinone mixture and poured into a 50mL small glass container (figure 1c). This glass container is placed on the magnetic stirrer and stirred continuously for 3 days at 350 to 450 rpm. A double beam spectrometer was used to take intermittent readings at 1,4,28,33 hours to calculate the amount of light of a given wavelength absorbed by an analyte in the sample. 3 days later the solutions are placed in a centrifugal machine for 10 minutes. Finally, the new nano formulation is collected in a plastic container for further test activities.

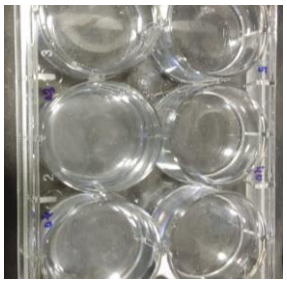


1a. 0.010 g of black cumin hydroxyapatite powder 1b. Mixed in 10mL of distilled water 1c. Thymoquinone mixture

Figure 1. Incorporation of blackcumin- hydroxyapatite crystal particles with thymoquinone

Cytotoxicity test

In 1 ml of distilled water, 0.1g of nanoparticle sample is combined. The samples are combined in various concentrations (10l, 20l, 30l, 40l, 50l) for cytotoxicity testing, with 10 nauplii in each concentration. The number of live nauplii is counted every 24 hours and the results are recorded.



(Cytotoxicity test well plates)

Figure 2. Cytotoxicity test done for black cumin- hydroxyapatite crystal particles with thymoquinone in different concentrations 10µl, 20µl, 30µl, 40µl, 50µl with 10 nauplii in each well

Media preparation

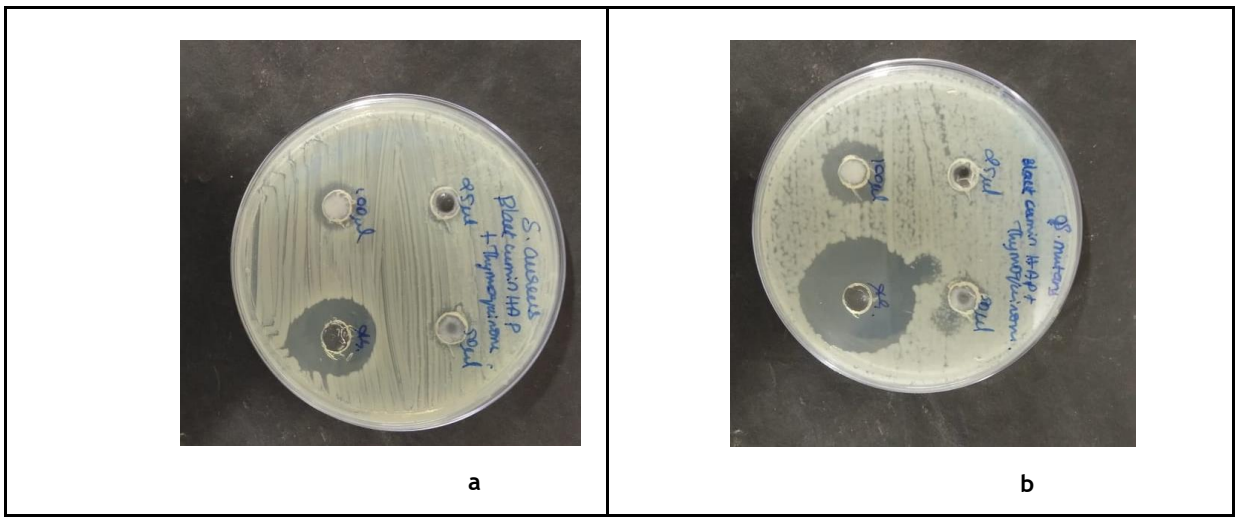
Mueller Hinton agar was prepared, sterilised, and poured onto Petri plates for Streptococcus mutans, Staphylococcus aureus, E. faecalis, and SDA agar for Candida albicans. The plates were given time to solidify. The antimicrobial efficacy was determined using the Agar Well Diffusion process.

Swabbing

After **solidification**, the oral pathogens Streptococcus mutans, Staphylococcus aureus, Enterococcus faecalis, and Candida albicans were swabbed on the respective plates.

Well formation

After swabbing, a gel puncher was used to create three wells on each plate. Green synthesised black cumin and hydroxyapatite particles solution in concentrations of 25 L, 50 L, and 100 L were loaded into those three wells. For Streptococcus mutans, Staphylococcus aureus, E.Faecalis, and Candida albicans, the plates were incubated at 37°C for 24 hours and 48 hours, respectively. The zone of inhibition was assessed and estimated after incubation.



a b

Figure 2: The antimicrobial activity test of green synthesized blackcumin-hydroxyapatite particle with thymoquinone against Streptococcus mutans (a) and Staphylococcus aureus (b)

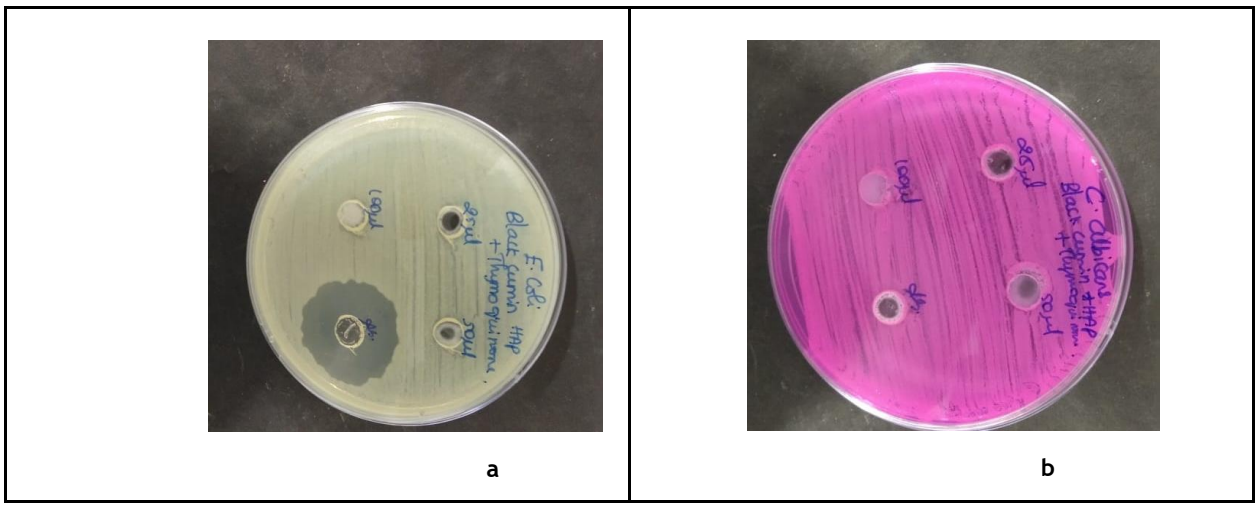


Figure 3: The antimicrobial activity test of green synthesized blackcumin-hydroxyapatite particle with thymoquinone against E. faecalis (a) and Candida albicans (b)

RESULTS

The cytotoxicity findings were described in table 1. The findings show that on day one, all of the nauplii in the well plate were alive. On day 2, however, in the 5l conc., 10 people were alive, in the 10l conc., 9 people were alive, and in the 20l, 40l, and 80l conc., 9, 8, 8 people were alive, respectively. Table 2 shows the diameter of the inhibition zone for all bacterial strains measured at various concentrations of green synthesised blackcumin and hydroxyapatite particles. Figure 2 shows the antimicrobial activity of nano formulation against Streptococcus mutans and Staphylococcus aureus. Zone of inhibition against Streptococcus mutans was found at 25 µL was 9 mm, 50 µL was 13 mm and at 100 µL was 18 mm. Zone of inhibition against Staphylococcus aureus at 25 µL was 9 mm, at 50 µL was 9 mm and at 100 µL was 14 mm. Figure 3 depicts the antimicrobial activity of new nano formulation against E. faecalis and Candida albicans. Zone of inhibition against E. faecalis at 25 µL was 9 mm, 50 µL was 9 mm and at 100 µL was 9 mm. Zone of inhibition against Candida albicans at 25 µL was 9 mm, 50 µL was 9 mm and at 100 µL was 9 mm. As a result, as the concentration of the new nano formulation increased, so did the antimicrobial activity.

Table 1. Cytotoxicity test of green synthesized black cumin-hydroxyapatite crystals with thymoquinone (no of live nauplii in the end of day1 and day2)

Concentration	Day1	Day2
5µl	10	10
10µl	10	9
20µl	10	9
40µl	10	8
80µl	10	8

(Counts of live nauplii in day1 and day2)

Table 2. Antimicrobial activity test and zone of inhibition of pathogens against the new nano formulation solution.

Name of the pathogen	Zone of inhibition			
	25µl	50µl	100µl	Ab
S.mutans	9mm	13mm	18mm	30mm
S.aureus	9mm	9mm	14mm	22mm
E.faecalis	9mm	9mm	9mm	30mm
C.albicans	9mm	9mm	9mm	12mm

(Zone of inhibition given in millimeter)

DISCUSSION

The mouth is colonised by 200 to 300 bacterial species, but only a small number of these species participate in dental caries or periodontal disease (18). N. sativa and its key component, thymoquinone, have been studied in depth due to their rich and complex chemical composition. In folk medicine, N. sativa has long been used to encourage good health and cure disease (19).

Our findings revealed that black cumin-hydroxyapatite crystals synthesized with thymoquinone have antimicrobial activity against Streptococcus mutans and Staphylococcus aureus. Antimicrobial activity against E. faecalis is absent and it is not effective against it. Antifungal activity against Candida albicans is effective and comparable to that of the antibody. The cytotoxicity test of black cumin-hydroxyapatite synthesized with thymoquinone shows that it is safe to use in all the concentration as a prescription drug for further research.

Recent research suggests that N. sativa extracts, especially thymoquinone, have a wide range of therapeutic effects, including antioxidant, anti-inflammatory, antimicrobial, anti-tumor, immunomodulatory, bronchodilatory, hypotensive, antidiabetic, hepatoprotective, gastroprotective, antihistaminic, and neuroprotective properties. In the current investigation, the new nano formulation tested against the dental pathogens that resulted in increased antibacterial activity against S. mutans and S. aureus at 100µl concentration. The current study does not evaluate the anti-inflammatory activity but according to author Tekeoglu et al. investigated the anti-inflammatory effects of thymoquinone on arthritis in rat models, finding that it reduced adjuvant-induced arthritis in rats (20). TNF-a and IL-1b levels in the thymoquinone-treated group were also significantly lower than in the control group, according to the researchers. Houghton et al. looked into the mechanisms of thymoquinone's anti-inflammatory activity, finding that it prevented thromboxane and leukotriene B4 synthesis from eicosanoids by inhibiting the cyclooxygenase and lipoxygenase enzymes (15).

The limitations of the study are that it is an in vitro study where we assessed the effect in the short term. We did not evaluate the anti-inflammatory and antioxidant properties of the new nano formulation. Our team has extensive knowledge and research experience that has translated into high quality publications (21–30) .Further research is needed to learn more about this new nano formulation's anti-inflammatory and antioxidant properties.

CONCLUSION

In conclusion the study showed that there is potential antimicrobial property of the new nano formulation against Streptococcus mutans and Staphylococcus aureus also there is antifungal activity against Candida albicans. Hence it can be prepared and formulated to use as mouth wash for the prevention of oral disease.

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