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Antimicrobial and Antioxidant Activities of Black Cumin Seed (*Nigella sativa*) Ethanol Extract

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ABSTRACT

Oral health faces significant challenges due to increasing dental infections by pathogenic microbes such as *Porphyromonas gingivalis, Streptococcus mutans*, and *Candida albicans*. Topical antioxidants in toothpaste, gel, and mouthwash are often used to treat dental diseases. There is a growing interest in finding natural alternatives for oral hygiene without side effects compared to conventional toothpaste formulations. *Nigella sativa* (black cumin) has various pharmaceutical efficacy, making it a valuable plant-based source medicinal compound. This study evaluated black cumin ethanol extract's (BCSE) antimicrobial and antioxidant activities. Antibacterial effectiveness was evaluated through Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), and disc diffusion assays against *P. gingivalis, S. mutans*, and *C. albicans*. Antioxidant activity was reported based on DPPH scavenging, ABTS reduction, H_2O_2 scavenging, and NO scavenging assays. Black cumin seed ethanolic extract (BCSE) showed antibacterial activity of 4.49 mm, 4.33 mm, and 7.12 mm for *P. gingivalis, S. mutans*, and *C. albicans*, respectively, with zones of inhibition increasing in a concentration-dependent manner ($p<0.05$). MIC and MBC evaluations also revealed that MIC for *S. mutans* was achieved at 12.5% BCSE concentration, while *P. gingivalis* and *C. albicans* required 25%, and MBC reached 100%. The IC_{50} for DPPH, ABTS, NO, and H_2O_2 were 25.41, 69.93, 87.18, and 95.16 µg/ml, respectively. The antioxidant activity increased in a concentration-dependent manner, especially at 100 µg/ml of BCSE. Based on these results, BCSE could be an optional ingredient in toothpaste.

1. Introduction

In recent years, dental infections caused by bacterial pathogens, including *Porphyromonas gingivalis, Streptococcus mutans*, and *Candida albicans*, have become a prevalent concern, posing significant challenges to oral health. These infections, which can lead to conditions such as gingivitis, periodontitis, and oral candidiasis, not only result in discomfort and pain but also contribute to the progression of more severe dental issues if left untreated (Mekhemar *et al.* 2020). Free radicals are exceedingly receptive particles that can cause oxidative stretch, harm verbal tissue, and trigger some dental issues (Carnelio *et al.* 2008). Topical cancer prevention agents within the frame of toothpaste, gel, and mouthwash are regularly utilized as a treatment for dental maladies (Palombo 2011; Kumar *et al.* 2022). In addressing this issue, there is increasing interest in exploring alternative and natural solutions for oral hygiene that do not cause side effects compared to conventional toothpaste formulations (Shaikh & Kumar

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2017). One promising approach is the incorporation of plant-derived ingredients into toothpaste formulations, leveraging the antimicrobial and antioxidant properties of botanical compounds.

The black cumin seeds (*Nigella sativa*), also recognized as black cumin, are a plant that encompasses a long history of restorative utilization. In conventional medication, these seeds are utilized to treat different afflictions, such as respiratory tract infections, stomach-related issues, kidney and liver problems, cardiovascular system disorders, and immune system support (Goreja 2003). Recent research shows that black cumin seeds contain various active compounds, such as thymoquinone, which has antibacterial, anti-inflammatory, antioxidant, fungicide, nephroprotective, hepatoprotective, and anticancer properties (Ahmad *et al.* 2021). The antibacterial movement of black cumin seeds has been broadly considered in advanced inquiries as a universal remedy for various bacterial infections (Bakathir & Abbas 2011).

Black cumin seed antibacterial activities have been extensively studied in advanced research, showing its potential as a natural cure for different bacterial diseases (Ketenoglu *et al.* 2020). This compound has been recognized for its ability to combat oxidative stress, which is linked to various chronic diseases. Khan & Kour (2016) also reported significant dose-dependent antibacterial impacts within the ethanol and *n*-hexane extracts derived from black cumin seeds against different gram-negative and gram-positive bacterial strains, including *Bacillus cereus, B. subtilis, Staphylococcus epidermidis, Escherichia coli, Salmonella typhimurium*, and *Klebsiella pneumonia*. The presence of bioactive compounds like thymoquinone in black cumin is believed to contribute to these antimicrobial effects, enhancing its potential as a natural remedy for oral health (Hannan *et al.* 2021). Black cumin also plays a vital role in oral health. Antioxidants help to mitigate oxidative stress, which is linked to inflammation and tissue damage in the oral cavity. The ability of black cumin to reduce inflammation and oxidative stress may further support its use in maintaining oral hygiene and preventing periodontal diseases (Ratheesh *et al.* 2021). Black cumin seeds display potential as antibacterial specialists, demonstrating the need for further exploration and validation of these properties. While there is limited direct evidence linking black cumin to anti-plaque effects, its recognized antibacterial and antioxidant qualities, as well as its traditional use in health promotion, suggest potential benefits. This considers points to assess the antibacterial action of black cumin seed ethanol extract (BCSE) against *P. gingivalis, S. mutans*, and *C. albicans* as well as antioxidant scavenging activity 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-Azino-bis-3-ethylbenzothiazoline-6-sulfonic corrosive (ABTS), Nitric oxygen (NO), and Hydrogen peroxide (H_2O_2) rummaging movement of BCSE.

2. Materials and Methods

2.1. Sample Preparation

The extraction procedure took place at PT Borobudur Industri Jamu (Natural Herbal Industry) Semarang, Indonesia, which is certified in Good Manufacturing Practices (GMP). The extraction of black cumin seeds (BCSE) was carried out using 70% ethanol solvent with the addition of excipients (maltodextrin) to produce a dry extract. The extract was then dissolved in DMSO into different concentrations (Laksmitawati *et al.* 2021). The BCSE concentrations used for antibacterial tests consist of 3.13%, 6.25%, 12.5%, 25%, 50%, 75%, and 100%. The concentrations used for antioxidant tests consist of 3.13 µg/ml, 6.25 µg/ml, 12.5 µg/ml, 25 µg/ml, 50 µg/ml, and 100 µg/ml.

The Indonesia Food and Drug Authority assessed BCSE quality in compliance with the guidelines outlined in Regulation No. 32 of 2019. This evaluation comprised organoleptic evaluation, physical characterization, and assessment of microbiological contamination.

2.2. Antimicrobial Sensitivity Test with Kirby-Bauer Disc Diffusion

The Kirby-Bauer disk dissemination strategy was utilized for antimicrobial affectability testing. Bacterial colonies were refined on Mueller Hinton Agar (MHA) medium, suspended in Mueller Hinton Broth (MHB) medium, and balanced to suitable turbidity to the McFarland standard of 0.5, creating around 1-2 × 10^8 CFU/ml. Each bacterial suspension was connected to the MHA surface utilizing sterile cotton. Paper plates (6 mm breadth) were splashed in different concentrations of test and control arrangements, and, at that point, were put on the agar surface. The experiment was repeated three times and incubated for 24 hours at 37°C. The inhibition zone diameter was measured using a caliper (Novilla *et al.* 2014).

2.3. Antimicrobial Sensitivity Test with MIC and MBC

The following antimicrobial test started with deciding the least inhibitory concentration (MIC) level utilizing the broth microdilution strategy. Before determining the MIC and MBC levels, each microorganism was prepared as an inoculum utilizing the direct colony suspension method. Inoculum was obtained by exchanging colonies of *S. mutans, P. gingivalis*, and *C. albicans* that had been developed for 24 hours on MHA media into Mueller Hinton Broth (MHB) (Himedia M391). Turbidity was calibrated to the McFarland 0.5 standard, which compared to 1-5 × 10^8 CFU/ml concentration. The determination of MIC levels was carried out on 96-well plates, each containing 100 µL of various extract (BCSE) concentrations of 100%, 75%, 50%, 25%, 12.5%, 6.25%, and 3.13%, supplemented with 100 µL of each microbe. The positive control utilized was 0.2% chlorohexidine, whereas the negative control was 10% DMSO. The plate was put into incubation and kept at 37°C for 24 hours. Then, the turbidity was assessed using spectrophotometry (Multiskan GO Thermo Scientific 51119300) in the 500-600 nm wavelength range. After absorbance measurement, the MBC level was determined by taking 100 µL and performing graded dilutions ranging from 10^2 to 10^5 in each MIC result well. Exactly 50 µL of the aqueous solution was cultured using the pour-plate technique on MHA agar. The plates were incubated for 24 hours at 37°C. The next day, we used a Funke Gerber 8500 colony counter to assess the number of bacterial colonies. The least bactericidal concentration (MBC) is characterized as the minimum concentration that produces an inhibitory effect of 99% (Balouri Moghaddam *et al.* 2016).

2.4. DPPH (2,2-Diphenyl-1-picrylhydrazyl) Scavenging Assay

Tests were conducted by adding as much as 50 µL of sample to the 96-well plate, and then 200 µL of 0.077 mmol DPPH was added. The clear wells received 250 µL of the test solution (DMSO 10%), while the control wells were supplemented with 250 µL of 0.077 mmol DPPH. After a 30-minute incubation at room temperature in the dark, the absorbance measurement at 517 nm wavelength was performed using a microplate reader. The following equation was utilized to calculate DPPH scavenging activity (Widowati *et al.* 2022a).

$$\text{DPPH Scavenging Activity (\%)} = \frac{\text{Control Absorbance} - \text{Sample Absorbance}}{\text{Control Absorbance}} \times 100 \quad (1)$$

2.5. ABTS (2,2'-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) Scavenging Assay

Tests were carried out by adding as much as 2 µL into a 96-well plate, and then 198 µL of ABTS reagent (Sigma Aldrich, A1888) was added. Blank wells were subjected to 200 µL of 10% DMSO, while control wells received 198 µL of ABTS reagent. The microplate was brooded at 37°C. In Absorbance, the wavelength was measured at 745 nm by employing a microplate peruser. The following equation is utilized to calculate ABTS scavenging activity (Prahastuti *et al.* 2020; Lister *et al.* 2019; Widowati *et al.* 2022b).

$$\text{ABTS Scavenging Activity (\%)} = \frac{\text{Control Absorbance} - \text{Sample Absorbance}}{\text{Control Absorbance}} \times 100 \quad (2)$$

2.6. Hydrogen Peroxide (H_2O_2) Scavenging Assay

A total of 60 µL of sample, 12 µL of 1 mM ferric ammonium sulfate (Merck, 1.03792.1000), and 3 µL of 5 mM H_2O_2 (Merck, 1.08597.1000) were added to a 96-well plate. Blank wells were treated with 90 µL of 10% DMSO, while negative control wells received 12 µL of ferric ammonium sulfate and 63 µL of 10% DMSO. The mixture was incubated for 5 minutes in the dark at room temperature. Subsequently, 1,10-phenanthroline (Merck, 1.07223.0010) 75 µL was added to both the test and control wells, followed by an additional incubation for 10 minutes in the dark at room temperature. The absorbance measurement was performed at 510 nm wavelength. The H_2O_2 scavenging activity was calculated utilizing the following formula (Prahastuti *et al.* 2020; Lister *et al.* 2019; Widowati *et al.* 2022b).

$$H_2O_2 \text{ Scavenging Activity (\%)} = \frac{\text{Control Absorbance} - \text{Sample Absorbance}}{\text{Control Absorbance}} \times 100 \quad (3)$$

2.7. Nitric Oxygen (NO) Scavenging Assay

Samples (10 µL) were added to 96-well plates, followed by 40 µL of sodium nitroprusside (Merck, 1065410100) in phosphate buffered saline (PBS) (Biowest, X0520-500). Blank wells were treated with 140 µL of 10% DMSO, while control wells received 10 µL of 10% DMSO. The mixture was incubated for 2 hours at room temperature. Then, 100 µL of Griess reagent was added, consisting of a 1:1 proportion of 1% sulfanilamide [Merck, 1117990100] in 2% H_3PO_4 [Merck 100573] and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride [Merck,

1062370025]. The absorbance measurement was performed at 546 nm wavelength. The NO scavenging activity of the samples was calculated using the following formula (Laksmitawati *et al.* 2021).

$$\text{NO Scavenging Activity (\%)} = \frac{\text{Control Absorbance} - \text{Sample Absorbance}}{\text{Control Absorbance}} \times 100 \quad (4)$$

2.8. Statistical Analysis

Data were represented as means and standard deviations. The analysis of variance (ANOVA) was utilised to compare different treatments, with $p<0.05$ set as the threshold for statistical significance. Subsequently, the Tukey HSD post-Hoc test and Dunnett's T3 test were performed. The median inhibitory concentration, or IC_{50} was calculated utilizing the probit method. All data were analyzed using IBM SPSS Statistics version 20.0.

3. Results

3.1. BCSE Quality

The evaluation of BCSE quality included tests for organoleptic properties, physical characteristics, and microbiological contamination. The results from these tests indicated that the quality of BCSE met the standards specified in Regulation No. 32 of 2019 issued by the Indonesian Food and Drug Authority, particularly concerning the safety and quality requirements for traditional medicines, as depicted in Table 1.

3.2. Antimicrobial Sensitivity by Disk Diffusion Test

Black cumin seed ethanol extract (BCSE) demonstrated antibacterial activity against *P. gingivalis, S. mutans,* and *C. albicans*, as indicated by the concentration-dependent diameter of the inhibition zone ($p<0.05$) (Figure 1). The largest inhibition zone was observed at the 100% BCSE concentration, with an average diameter of 4.49 mm, 4.33 mm, and 7.12 mm for *P. gingivalis, S. mutans,* and *C. albicans*, respectively.

3.3. Antimicrobial Sensitivity by MIC and MBC Test

The antibacterial evaluation using MIC and MBC methods is presented in Figures 2A and B. These methods demonstrated the effect of BCSE concentration on bacterial and fungal microorganisms. The MIC value of *S. mutans* (51.41%) was observed at a concentration of 12.5%, while for *P. gingivalis* (53.57%) and *C. albicans* (54.10%), they were observed at a concentration of 25% (Figure 2A and B). The MBC value of BCSE was found at a 100% concentration, showing a significant difference ($p<0.05$) according to the colony counts presented in Table 2.

3.4. DPPH Scavenging Activity

BCSE increased DPPH scavenging activity in a concentration-dependent manner, as shown in Figure 3. The highest DPPH scavenging activity

Table 1. BCSE quality based on certificate of analysis

Item	Specification	Test result	Test method
	Identification test		
Appearance	Granule	Complies	Visual
Color	Brown	Complies	Visual
Odor	Aromatic	Complies	Organoleptic
Taste	Bitter	Complies	Organoleptic
Mesh size	70% pass mess 12	Complies	12 mesh screen
Loss pn drying	5.0% max	2.57%	2 g/105°C/15 minutes
	Heavy metals		
Arsenic (As)	5 ppm max	Complies	AAS
Lead (Pb)	10 ppm max	Complies	AAS
	Microbiological test		
Total plate count	Not more than 1,000 cfu/g	<100 cfu/g	Dilution plating
Fungi/yeast and molds	Not more than 1,000 cfu/g	<100 cfu/g	Dilution plating
E. coli	Should be absent	Complies	MPN method
Salmonella	Should be absent	Complies	Dilution plating
S. aureus	Should be absent	Complies	Dilution plating
P. auruginosa	Should be absent	Complies	Dilution plating

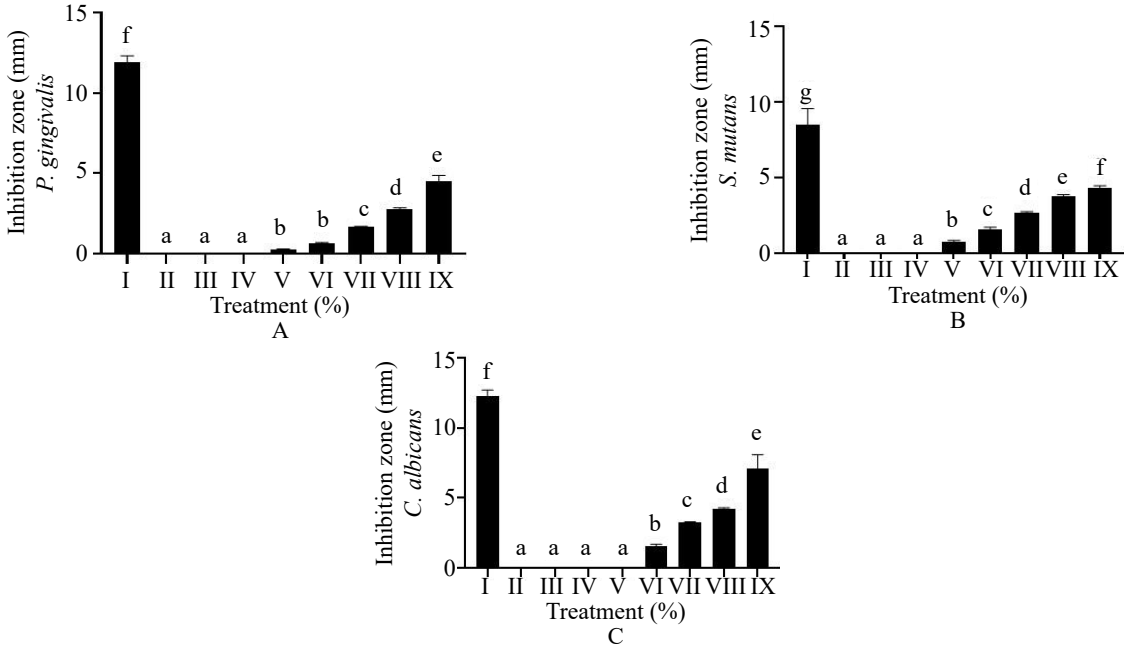

Figure 1. Antimicrobial activity of BCSE at various concentrations against (A) *P. gingivalis*, (B) *S. mutans*, (C) *C. albicans*. Data presented as mean ± SD. I: positive control (Chlorhexidine 0.2%); II: negative control (DMSO); III: BCSE 3.13%; IV: BCSE 6.25%; V: BCSE 12.5%; VI: BCSE 25%; VII: BCSE 50%; VIII: BCSE 75%; IX: BCSE 100%. The experiment was repeated three times for each concentration level, ensuring a comprehensive and reliable assessment. Various letters (a, b, c, d, e, f, and g) showed significant differences among BCSE concentration ($p<0.05$)

was observed at a BCSE concentration of 100 µg/ml, reaching 87.81%. The BCSE's DPPH scavenging activity IC_{50} value was 25.41 µg/ml, indicating that 25.41 µg/ml concentration was required to inhibit 50% of DPPH radicals, classifying it within the category of very strong antioxidant.

3.5. ABTS Scavenging Activity

BCSE increased the ABTS scavenging activity in a concentration-dependent manner, as shown in Figure 4. The highest ABTS scavenging activity was observed at a BCSE concentration of 100 µg/ml, reaching 28.96%. The BCSE's ABTS scavenging activity IC_{50} value was 69.93 µg/ml, categorizing it as a strong antioxidant.

3.6. NO Scavenging Activity

BCSE increased NO scavenging activity in a concentration-dependent manner, as shown in Figure 5. The highest NO scavenging activity was observed at a BCSE concentration of 100 µg/ml, reaching 56.95%. The BCSE's NO scavenging activity IC_{50} value was 87.18 µg/ml, categorizing it as a solid antioxidant.

3.7. H_2O_2 Scavenging Activity

BCSE increased H_2O_2 scavenging activity in a concentration-dependent manner, as depicted in Figure 6. The highest H_2O_2 scavenging activity was observed at a BCSE concentration of 100 µg/ml, reaching 52.56%. The BCSE's H_2O_2 scavenging activity IC_{50} value was 92.16 µg/ml, classifying it as a strong antioxidant.

4. Discussion

Nigella sativa, or black cumin seed, has been studied for its antibacterial properties against various microorganisms. Black cumin seed ethanol extract (BCSE) has shown antibacterial action against *P. gingivalis, S. mutans*, and *C. albicans*, with the diameter of the inhibition zones increasing with the extract concentration (Figure 1). Studies have shown that *N. sativa* seed extract exhibits concentration-dependent inhibition against pathogenic yeast of *C. albicans*, Gram-negative bacteria such as *Pseudomonas aeruginosa* and *Escherichia coli*, and Gram-positive bacteria such as *Staphylococcus aureus* (Hanafy & Hatem 1991). Additionally, a study found that *N. sativa* had significant antibacterial activity against *P. intermedia*

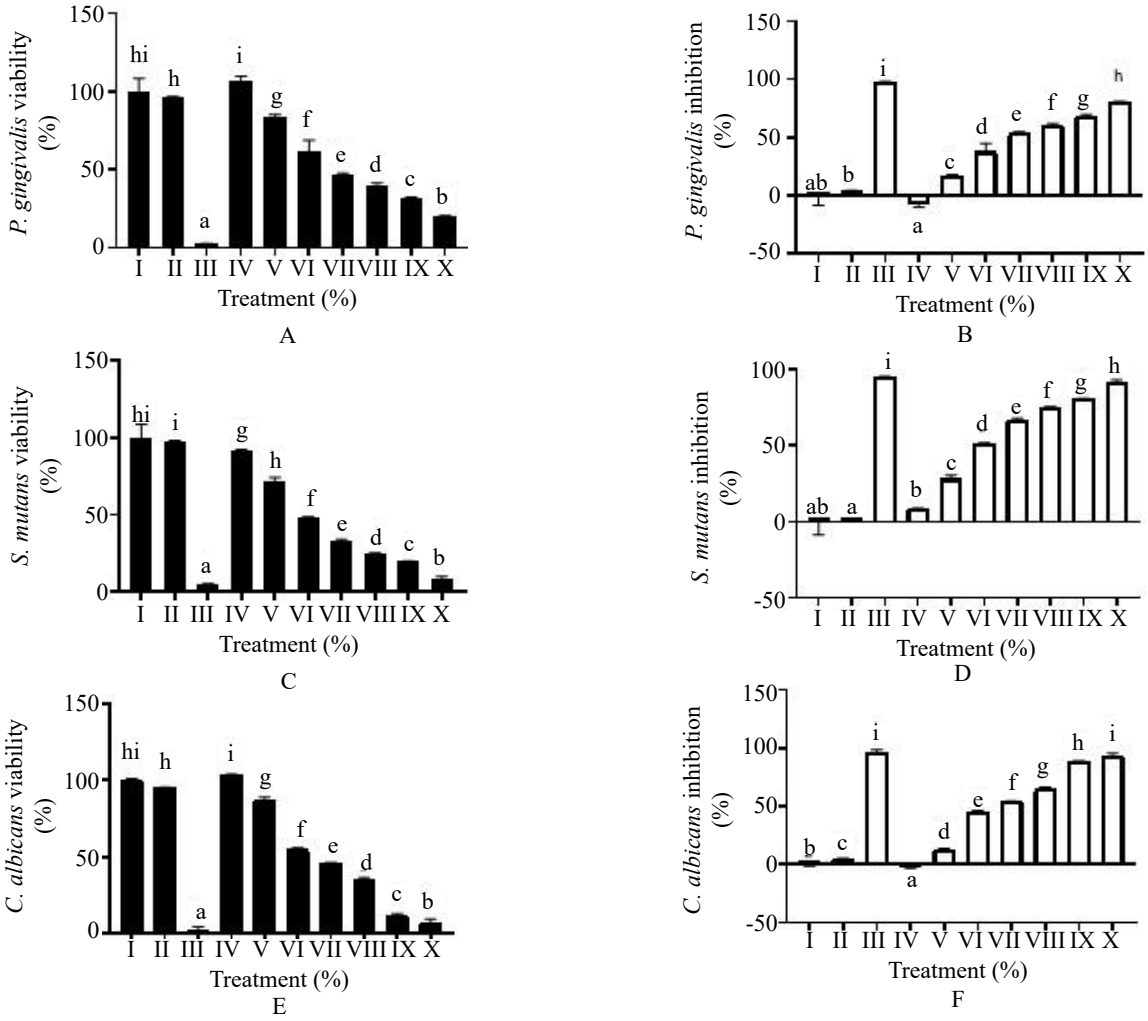

Figure 2. Antimicrobial activity of BCSE at various concentrations against various bacteria with MIC and MBC tests (*S. mutans*; *P. gingivalis*; *C. albicans*). Data presented as mean ± SD. I: growth control; II: negative control (DMSO); III: positive control (Chlorhexidine 0.2%); IV: BCSE 3.13%; V: BCSE 6.25%; VI: BCSE 12.5%; VII: BCSE 25%; VIII: BCSE 50%; IX: BCSE 75%; X: BCSE 100%. The assay was performed three times for each concentration level to ensure accuracy and reliability. Various letters showed significant differences among BCSE concentration ($p<0.05$)

Table 2. Effect of BCSE on average colony counts of *S. mutans, P. gingivalis*, and *C. albicans*

Sampel	CFU/ml		
	S. mutans	*P. gingivalis*	*C. albicans*
GC	TNTC	TNTC	TNTC
NC	TNTC	TNTC	TNTC
PC	0	0	0
BCSE 3.13%	TNTC	TNTC	TNTC
BCSE 6.25%	TNTC	TNTC	TNTC
BCSE 12.5%	219.67×10^4	239.67×10^4	180.00×10^4
BCSE 25%	143.67×10^4	158.00×10^4	130.33×10^4
BCSE 50%	68.33×10^4	92.33×10^4	91.00×10^4
BCSE 75%	33.33×10^4	57.00×10^4	7.00×10^4
BCSE 100%	0	14.33×10^4	0

GC: growth control, NC: negative control (DMSO), PC: positive control (Chlorhexidine 0.2%), BCSE (Black curcumin seed extract) at 3.13%, 6.25%, 12.5%, 25%, 50%, 75%, 100%; TNTC: To Numerous Too Count >250 colonies

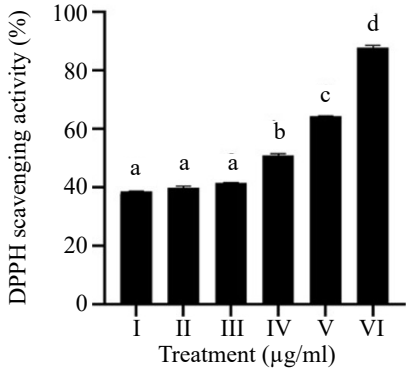

Figure 3. Antioxidant movement of BCSE at different concentrations against DPPH scavenging activity. Data presented as mean ± SD. I: 3.13 µg/ml; II: 6.25 µg/ml; III: 12.5 µg/ml; IV: 25 µg/ml; V: 50 µg/ml; VI: 100 µg/ml. The measurement was repeated three times for each concentration to ensure accuracy and reliability. Various letters showed significant differences among BCSE concentration ($p<0.05$)

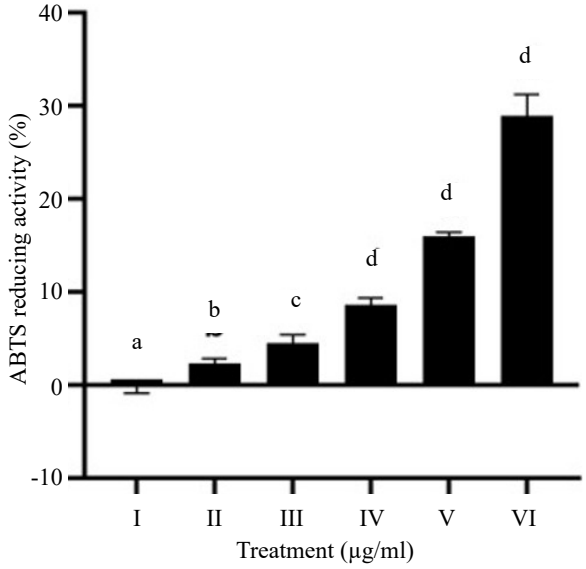

Figure 4. Antioxidant movement of BCSE at different concentrations against ABTS scavenging activity. Data presented as mean ± SD. I: 3.13 µg/ml; II: 6.25 µg/ml; III: 12.5 µg/ml; IV: 25 µg/ml; V: 50 µg/ml; VI: 100 µg/ml. The measurement was repeated three times for each concentration to ensure accuracy and reliability. Various letters showed significant differences among BCSE concentration ($p<0.05$)

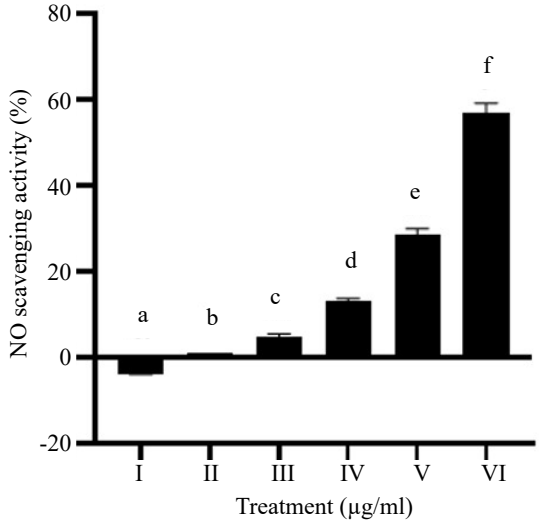

Figure 5. Antioxidant movement of BCSE at different concentrations against NO scavenging activity. Data presented as mean ± SD. I: 3.13 µg/ml; II: 6.25 µg/ml; III: 12.5 µg/ml; IV: 25 µg/ml; V: 50 µg/ml; VI: 100 µg/ml. The measurement was repeated three times for each concentration to ensure accuracy and reliability. Various letters showed significant differences among BCSE concentration ($p<0.05$)

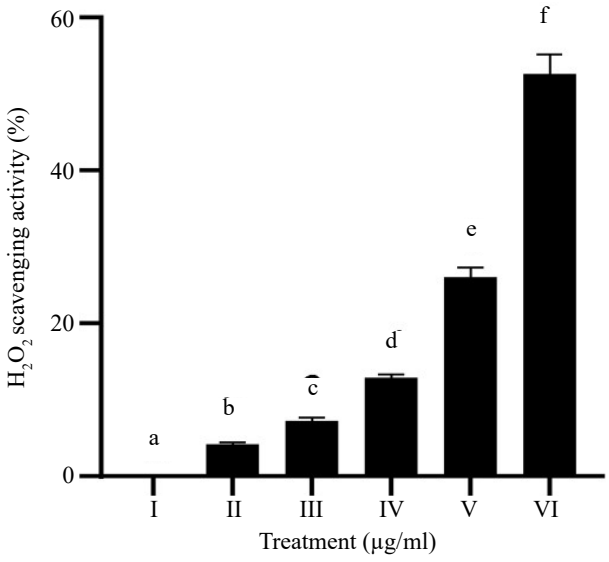

Figure 6. Antioxidant action of BCSE at different concentrations against H_2O_2 scavenging activity. Data presented as mean ± SD. I: 3.13 µg/ml; II: 6.25 µg/ml; III: 12.5 µg/ml; IV: 25 µg/ml; V: 50 µg/ml; VI: 100 µg/ml. The measurement was repeated three times for each concentration to ensure accuracy and reliability. Various letters showed significant differences among BCSE concentration ($p<0.05$)

and *P. gingivalis*, two significant periodontal pathogens (Senthilnathan *et al.* 2020). The antibacterial activity of black cumin seeds is due to their chemical constituents, particularly thymoquinone and melanin. Thymoquinone, one of the most active constituents of *N. sativa*, has been reported to have notable antimicrobial impacts against a wide range of microorganisms, including Gram-positive and Gram-negative bacteria, fungi, and viruses (Forouzanfar & Hosseinzadeh 2020). Thymoquinone inhibits bacterial activity through several mechanisms, including bactericidal activity, biofilm inhibition, and reactive oxygen species (ROS) generation (Goel & Mishra 2018).

Figures 2A-F show the results of the three bacteria that cause tooth decay when affected by the BCSE concentration. This can be caused by the concentration level of the chemical substances contained in the extract (Helmiyanti 2010). Other research suggests that concentration levels influence the environmental conditions, creating an unfavorable environment for bacterial growth. An inappropriate pH can lead to

bacterial inhibition, which also affects the nutrient content needed by bacteria, potentially reducing it (Fajar *et al.* 2022). Many studies report the phytochemical content of black cumin, namely tannins, terpenoids, steroids, alkaloids, as well as phenolic compounds and derivatives (Anam *et al.* 2021). Other compounds with antibacterial properties include thymoquinone, carvacrol, alpha-hederin, and nigellimine-N-oxide, which also exhibit anti-inflammatory and antibacterial effects (Callixte *et al.* 2021). These complex compounds form mechanisms that kill bacteria by interacting with extracellular proteins, which can damage bacterial membranes and interfere with the formation of peptidoglycan (Amalia *et al.* 2018; Saptowo & Supriningrum 2022).

This research shows that BCSE exhibits antioxidant activity, which was measured by various assays such as DPPH, ABTS, NO, and H_2O_2 scavenging activities (Figures 3, 4, 5, and 6, respectively). In this study, all antioxidant test results indicate an increase in scavenging activity with the addition of BCSE concentration. This indicates that the higher the compound concentration, the stronger its ability to capture or inhibit free radicals or oxidative activity. This result is supported by Gueffai *et al.* (2022), who reported that black cumin seeds exhibited a total phenolic content ranging from 19.2 to 35.6 mg GAE/g and a DPPH radical scavenging activity ranging from 35 to 70.5%. The black cumin seed antioxidant properties arise from their high total phenolic content and the presence of bioactive phenolic compounds. Among these compounds, flavonoids such as flavonol triglycosides, including quercetin and flavonoid rutin, contribute significantly to the antioxidant capacity of black cumin seeds (Rusmarilin *et al.* 2019).

The DPPH assay is employed to assess the ability of antioxidant molecules to scavenge free radicals in DPPH solutions. DPPH is considered a stable free radical due to its electrons being delocalized throughout the molecule, which prevents dimerization, a characteristic uncommon among free radicals (Widowati *et al.* 2022a). As an antioxidant substance interacts with DPPH, it leads to the decrease of free radicals, evident by the observable shift from a purple hue to yellow. The ABTS reduction assay assesses the antioxidant compounds to neutralize the free radical activity ability of the diammonium salt ABTS+, which is generated by reacting ABTS with a strong oxidizing agent. The resulting greenish-blue solution is then reduced by antioxidants that donate hydrogen (Widowati *et al.* 2018). The basis of the NO scavenging assay lies in the fact that particular nitric oxide synthases initiate a biochemical process generating NO within biological tissues. When sodium nitroprusside reacts with oxygen in a buffer solution, nitrite ions are produced, the concentration of which can then be measured using Griess reagent (Alam *et al.* 2013). Among reactive oxygen (ROS) species, H_2O_2 holds significance, as it is not inherently toxic. However, it is frequently transformed into more harmful radicals, such as hydroxyl radicals (OH), through the Fenton reaction or into acids by the enzyme myeloperoxidase (Mukhopadhyay *et al.* 2016).

IC_{50} indicates the concentration of a substance required to mitigate free radical activity or oxidative processes by 50%. Therefore, a lower IC_{50} value signifies higher efficiency in capturing or inhibiting free radicals. This can be interpreted as the substance's ability to protect cells or tissues from damage caused by oxidative stress. The DPPH BCSE scavenging activity IC_{50} value was 25.41 µg/ml, which is categorized as a very strong antioxidant (Table 3). Meanwhile, the IC_{50} results from ABTS, NO, and H_2O_2 scavenging activities categorize BCSE as a strong antioxidant with an IC_{50} value of <100 µg/ml. Determined based on the IC_{50} value, the antioxidant control of a compound can be classified as exceptionally solid in the event that the IC_{50} value is <50 µg/ml, categorized as strong with an IC_{50} value between 50 and 100 µg/ml, moderate with an IC_{50} value between 100 and 150 µg/ml, and weak with an IC_{50} value between 151 and 200 µg/ml (Tidke *et al.* 2018). Thus, in brief, the mechanism that occurs in antioxidants and antibacterials contained in the extract will interact to increase the antibacterial effect with antioxidants that inhibit oxidation reactions, leading to the inhibition of nucleic acid protein synthesis, which reduces cell membrane function (Pelealu *et al.* 2021; Rahmawati *et al.* 2022).

In conclusion, black cumin seeds (BCSE) show antibacterial properties against *P. gingivalis, S. Mutans*, and *C. albicans*, as demonstrated by the disk diffusion test, MIC test, and MBC test. BCSE exhibits strong antioxidant activities against DPPH, ABTS, NO, and H_2O_2 with IC_{50} values of 25.41 µg/ml, 69.93 µg/ml, 87.18 µg/ml, and 92.16 µg/ml, respectively.

Table 3. The IC_{50} values of antioxidant activities of BCSE

Scavenging activity assays	Linear equation	R^2	IC_{50} (µL/ml)
DPPH	Y = 0.5184x + 36.829	0.99	25.41
ABTS	Y = 0.2913x + 0.5007	0.99	69.93
NO	Y = 0.6117x - 3.3258	0.99	87.18
H_2O_2	Y = 0.5265x - 0.1026	0.99	95.16

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