Antibacterial Activity of Ethanol Extract of *Nigella Sativa* L. Seed Against *Streptococcus mutans*

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Abstract. Dental caries or cavities are most commonly found in the oral cavity. Dental caries is caused by plaque-forming bacteria such as *Streptococcus mutans*. Getting dental care with a professional dental expert every two weeks or three-monthly intervals can prevent caries but it is expensive and difficult for most individuals. So an alternative is sought, especially in herbal plants. Black cumin (*Nigella sativa* L.) from the Ranunculanceae family has been used as a medicinal plant for various diseases for more than 2000 years. This study aims to determine the antibacterial effect of *Nigella sativa* L. seed ethanol extract on *Streptococcus mutans* and to find out at what concentrations *Nigella sativa* L. seed ethanol extract provides antibacterial activity. This research was carried out by extracting *Nigella sativa* L. seed using ethanol solvent. Activity tests are carried out using paper disc diffusion method. The results of inhibition zones obtained from concentrations of 400, 600, and 800 mg/mL of black cumin extract were 13.3, 16.4 and 20.3 mm, respectively. Furthermore, the minimum inhibitory concentration test (MIC) for *Streptococcus mutans* bacteria was obtained at a concentration of 380 mg/mL.

1. Introduction

Dental caries is a disease of the hard tissues of the teeth most commonly found in the oral cavity. Caries experience varies greatly between countries, depending on age, lifestyle, diet and socio-economic conditions. Results of Basic Health Research in 2013 in the field of dental and oral health showed that the active caries prevalence of the Indonesian population was 43.4% [1].

Dental caries or tooth decay is a progressive and irreversible microbial disease characterized by fermentation of bacterial carbohydrates that produce acid production causing destruction of hard tissue in the teeth. Among various oral microorganisms, *Streptococcus mutans* (*S. mutans*) has been identified as a plaque-forming bacteria that is capable of producing dental caries in humans. Several attempts have been made to eliminate *S. mutans* from oral flora. There is no convincing evidence that careful mechanical debridement by patients has reduced the incidence of tooth decay, even though the procedure can reduce the amount of dental plaque and inflammation level of the gums (inflammation of the gingiva or gum). However, if mechanical debridement is performed by a dental professional once every two weeks or quarterly intervals, and includes fluoride treatment, both decay and periodontal disease basically stop happening. The level of dental debridement that is carried out professionally is very labor intensive so that the cost is expensive and unable to afford by most individuals[2].

Black cumin (*Nigella sativa* L.) is from the Ranunculanceae family has been used as a medicinal plant for more than 2000 years. This plant has light purple or white flowers and the fruit is capsule shaped, with small white and trigonal seeds. Once cooked, the capsule opens and the seeds turn black [3]. *Nigella sativa* L. seed contains essential oils, saponins, polyphenols, fatty oils, fatty acids and bitter nigelon, negin and thymokinon substances. Black cumin seeds are
widely used as a medicine for various diseases. These seeds have been shown to strengthen the immune system, prevent blockages in blood vessels, reduce cholesterol, improve heart performance, improve memory and concentration, increase hormone bioactivity, neutralize toxins, overcome sleep and stress, warm the stomach, improve the digestive tract, anti-inflammatory, launch breast milk, rejuvenate skin cells and have anti-tumor activity [4]. Various studies have shown that *Nigella sativa* L. seed have activity against several bacteria such as *E. coli*, *P. aeruginosa*, *S. flexneri*, *S. typhimurium*, *S. enteritidis* and *S. aureus* [4]. In previous study performed *Nigella sativa* L. seed extracted with ethanol showed antibacterial activity against Methicillin resistant Staphylococcus aureus (MRSA) [4]. Therefore, this study was conducted to complement previous research by conducting antibacterial activity of ethanol extract of black cumin seeds against *S. mutans* causing dental caries. Therefore, the aim of this study was to determine total phenolic and total flavonoid contents in water, ethanol and chloroform extracts of *Baeckea frutescens* and also establish their potential effects on health promotion as antioxidant and antimicrobial agents.

2. Materials and Methods

2.1. Bacteria

*Streptococcus mutans* bacteria (ATCC 35668) was obtained from the Faculty of Dentistry, Padjadjaran University.

2.2. Preparation of *Nigella sativa* L. seed extracts

*Nigella sativa* L. seed was extracted using 96% ethanol by maceration method. The fine powder of black cumin seeds was dissolved in 96% ethanol solvent for 24 hours and the solvents were replaced every 24 hours for three times then a thick extract was obtained. Organoleptic and phytochemical screening of black cumin seed extract was carried out as a qualitative measurement in determining the secondary metabolites of *Nigella sativa* L. seed extract included with water content testing.

2.3. Antibacterial activity assay of *Nigella sativa* L. against *S. mutans*

Antibacterial activity of *Nigella sativa* L. against *S. mutans* was performed with agar diffusion method. Bacterial suspension of *S. mutans* with optical density which was equal to 0.5 Mc Farland standard was incubated in Muller Hinton Broth (MHB) at 37 °C for 24 h. The sterile cotton swab was dipped in bacterial suspension in several times press heavily on the inside wall of the tube above the fluid level. Bacteria was inoculated in the dried surface of a Mueller-Hinton agar (MHA) plate by streaking the swab over the entire sterile agar surface. Then, paper disc was put on the MHA dan loaded with 20 µL of 200 mg/mL, 400 mg/mL, 600 mg/mL and 800 mg/mL. Plate was inverted and incubated at 37 °C for 24 h. Antibacterial activity was determined by the inhibition zone formed around the disc paper containing extract of *Nigella sativa* L. seed. The inhibitory zone was determined in millimeters (mm) using the calipers [5].

2.4. Minimum Inhibition Concentration assay of *Nigella sativa* L. against *S. mutans*

Minimum inhibition concentration was performed with agar dilution plates. The extract was incorporated into the agar medium (MHA), with each plate containing a different concentration of the extract i.e. 360, 370, 380, 390 and 400 mg/mL. The inoculum can be applied rapidly and simultaneously to the agar surface. A total of 100 µL of 107 CFU/mL bacterial suspensions was placed on the surface of the agar (10 cm diameter Petri dish) and spread evenly using a spreader. Allow the inoculated plates to stand at room temperature until the moisture in the inoculum spots has been absorbed into the agar, until the spots are dry, but no more than 30 mins. Then, the plates were inverted and incubated at 35±2 °C for 18 h. MIC was noted as the lowest concentration that inhibit the growth of bacteria [6].
2.5. Equality test of Nigella sativa L. extract activity against amikacin in growth inhibition of S. mutans

This equality test used MHA media. A number of 100 uL of $10^7$ CFU/mL bacterial suspensions was evenly distributed on the surface of agar media. Inoculated plate was left at room temperature for no more than 30 min. Then, the paper discs were placed on the inoculated plate surface. The paper discs were 20 µL loaded with serial concentration of amikacin i.e. 100 µg/mL, 200 µg/mL, 300 µg/mL, 400 µg/mL and 500 µg/mL and Nigella sativa L. extract i.e. 400 mg/mL, 600 mg/mL, 800 mg/mL, 1000 mg/mL and 1200 mg/mL. The Petri dish was incubated 37 °C for 24 h. The diameters of the inhibiton zone were measured with the caliper.

3. Results and Discussion

3.1. Nigella sativa L seed extract

Organoleptic test result was performed in Table 1. Whereas the organoleptic of Nigella sativa L. seed oil extract showed yellowish brown, aromatic odor and agreeable taste [7]. Water content of Nigella sativa L. ethanol extract was 5% (v/v). The water content in the extract must not be more than 10% [8].

| Organoleptic properties | Blackish red | Bitter | Typical fragrance | Viscous liquid |
|-------------------------|--------------|--------|-------------------|---------------|
| Color                   |              |        |                   |               |
| Taste                   |              |        |                   |               |
| Odor                    |              |        |                   |               |
| Form                    |              |        |                   |               |

The ethanol extract of Nigella sativa L. seed contained the component some secondary metabolites i.e. alkaloids, tannin, terpenoids, saponin and steroids. Secondary metabolites such as alkaloids, tannin, terpenoids, saponin and steroid were also found in black seed of Nigella sativa L. [9].

3.2. Antibacterial activity assay of Nigella sativa L. against S. mutans

The Nigella sativa L. seed extract had performed bacterial activity with diameter of inhibition zones i.e 13.3±0.54, 16.4±0.75, and 20.3± 0.35 for extract concentrations 400, 600 and 800 mg/mL respectively ‘figure 1’. From this result to determine the MIC value, the concentration range was started between 200-400 mg/mL.

Figure 1. Antibacterial activity of Nigella sativa L. seed ethanol extract against S. mutans
3.3. Minimum Inhibition Concentration assay of Nigella sativa L. against S. mutans

MIC value of Nigella sativa L. seed extract was 380 mg/mL. At this concentration Nigella sativa L. seed extract inhibited the bacterial growth. The extracts that are from herb can be categorized as inhibitors of bacterial growth are extracts that have less than 100 mg/mL of MIC, moderate 100-500 mg/mL, weak 500-1000 mg/mL [10]. So, the extract of Nigella sativa L. seed had moderate bacterial activity to S. mutans. Whereas the diethyl ether extract of Nigella sativa L. seeds (25-400 µg extract/disc) had bacterial activity to Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Candida albicans [11].

**Tabel 2.** Minimum inhibitory concentration of Nigella sativa L. seed extract against S. mutans

| Extract concentration (mg/mL) | Growth of S. mutans |
|------------------------------|---------------------|
| 360                          | +                   |
| 370                          | +                   |
| 380                          | -                   |
| 390                          | -                   |
| 400                          | -                   |

+ = bacterial growth, - = no bacterial growth

**Tabel 3.** Equality test of Nigella sativa L. extract activity against amikacin in growth inhibition of S. mutans

| Extract concentration (mg/ml) | Log (C) | Diameter of inhibition zone (mm) | Amikacin concentration (µg/ml) | Log (C) | Diameter of inhibition zone (mm) |
|------------------------------|---------|----------------------------------|-------------------------------|---------|----------------------------------|
| 400                          | 2.6     | 13.8 ±0.61                       | 100                           | 2       | 13.8 ±0.61                       |
| 600                          | 2.78    | 16.8 ±0.32                       | 200                           | 2.3     | 16.8 ±0.32                       |
| 800                          | 2.9     | 21.9 ±0.55                       | 300                           | 2.48    | 21.9 ±0.55                       |
| 1000                         | 3       | 24.8 ±0.55                       | 400                           | 2.6     | 24.8 ±0.55                       |
| 1200                         | 3.08    | 26.6 ±0.66                       | 500                           | 2.7     | 26.6 ±0.66                       |

\[ y = 26.2x-54.1 \quad r^2 = 0.9951 \]
\[ y = 13.4x-14.7 \quad r^2 = 0.9936 \]

3.4. Equality test of Nigella sativa L. extract activity against amikacin in growth inhibition of S. mutans

Equality assay of Nigella sativa L. seed extract activity compared to Amikacin performed that 1 part of extract activity was equal to 3 x 10-4 part of Amikacin. This revealed that extract activity was still lower than Amikacin activity.

4. Conclusion

The ethanol extract of Nigella sativa L. seed had bacterial activity against S. mutans with MIC value i.e. 380 mg/mL and equality of activity of 1: 3x10-4 against Amikacin.
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