ANTIBACTERIAL ACTIVITY OF BLACK CUMIN (NIGELLA SATIVA) EXTRACT AGAINST STREPTOCOCCUS SANGUINIS (ATCC) BACTERIA

Syahdiana Waty[1], Yetti Lusiani[2], Zulfa Ismaniar Fauzi[3], Lisda Rimayani Nasution[4], Nurul Hidayah[5], Zanzabillah Rambe[6]
[12356]Politeknik Kesehatan Kementerian Kesehatan Medan
[4]Universitas Sumatera Utara
Email : [1]syahdianawaty@gmail.com

ABSTRACT

Streptococcus sanguinis bacteria are one of the primary causes of dental caries and periodontal disease. Black cumin is suspected to contain secondary metabolites including flavonoids, alkaloids, thymoquinone, saponins, tannins, steroids and phenols, which possess potential antibacterial properties. This study was a true experiment using a post-test only control group design, aiming to determine the in vitro antibacterial activity of black cumin extract against Streptococcus sanguinis bacteria. Samples were tested at concentrations of 25%, 50%, 75% and compared with a positive control (0.2% chlorhexidine) and a negative control (Dimethyl Sulfoxide/ DMSO). Data were analyzed using ANOVA and Post Hoc (Tukey) tests. The results showed an average inhibition zone in the moderate category at concentrations of 25% (10.63 mm), 50% (10.66 mm), and 75% (10.9 mm). The ANOVA test revealed a significant difference between the groups, with a p-value of 0.061 ($p < 0.1$). The Post Hoc (Tukey) test indicated that the 75% concentration of black cumin extract had comparable antibacterial potential to chlorhexidine ($p > 0.1$). This study concluded that black cumin extract can significantly inhibit Streptococcus sanguinis bacteria at a 75% concentration.

Keywords : *Black-Cumin Extract; Streptococcus sanguinis; Caries*

INTRODUCTION

Oral and dental health is the condition of hard and mild system in health oral cavity and free from diseases or disorders that may affect appearance. Thus, individual do not have difficult in speaking, consuming food and interacting with others (Sumadewi & Harkitasari, 2023). According to the 2023 Indonesian Health Survey (SKI) that prevalence of dental caries in Indonesia is 43.9%. Among children aged 5-9 years, the prevalence is 49.9% and 5.3% among children aged 10-14 years. These statistics indicate that the incidence of dental caries in Indonesia remains high, while proper tooth brushing behavior is still very low. Therefore, education and further research related to oral health are necessary.

Dental plaque also affect caries formation as it consists of clusters bacteria that proliferate and firmly attach to tooth surfaces in individual which ignore oral hygiene (Zakki, 2017). Dental calculus, formed from accumulated plaque, provides nutrients for bacterial colonies that produce acids and damage enamel (Prasko *et al*., 2022). There are approximately 700 bacterial species found in the oral cavity, including plaque-forming bacteria (Kusuma, 2016). *Streptococcus mitis*, *Streptococcus oralis*, and *Streptococcus sanguinis* bacteria are among the first bacterial groups to adhere the acquired pellicle during dental plaque formation (Zhu *et al*., 2018). *Streptococcus sanguinis* facilitates colonization by other bacteria as it can initiate bonding with other species in the oral cavity, such as *Streptococcus gordonii*, *Actinomyces naeslundii*, *Streptococcus mutans*, and *Prevotella loescheii* (Syahrani *et al*., 2024). There are two main methods of plaque control viz mechanically, through flossing and toothbrushing or chemically such as rinsing with mouthwash to prevent food debris from adhering to the enamel (Ristianti *et al*., 2015). Although effective, alcohol based mouthwashes may cause adverse effects, such as dry mouth, reduced salivary flow, halitosis, and an increased risk of enamel erosion and tooth damage (Asridiana & Thioritz, 2020).

Chlorhexidine is widely recognized as the most effective chemical agent for plaque control due to its prolonged antimicrobial activity. However, long term use may cause adverse effects such as tooth and tongue discoloration, altered taste perception, and disruption of the natural oral microbiota, which can lead to opportunistic bacterial overgrowth (Sari *et al*., 2020). These drawbacks have prompted growing interest in safer, plant based alternatives. Herbal mouthwashes made from natural extracts approach to minimize side effects while maintaining

antimicrobial efficacy, such as cinnamon bark have demonstrated effectiveness in reducing dental plaque (Waty et al., 2023),

The study conducted by Zuraida *et al*. (2022) reported that *Nigella sativa* extract successfully inhibited the growth of *Staphylococcus aureus* at all tested concentrations. This finding is consistent with a previous study by Makmun *et al*. (2020), which also demonstrated inhibition zones against *S. aureus* at all concentrations of *Nigella sativa* extract. Furthermore, antibacterial activity testing of black cumin seeds revealed inhibition of *Streptococcus mutans* growth, with inhibition zones ranging from 0.83 mm at the lowest concentration (3%) to the highest concentration (6%) (Satrio *et al*., 2020). Therefore, the researcher is interested in conducting this study to determine the antibacterial activity of black cumin extract against *Streptococcus sanguinis*.

METHOD

This study employed a true experimental design using the post test only control group design, which involves conducting observations or measurements after treatment and comparing the results with a control group. The antibacterial activity of black cumin extract (*Nigella sativa*) against *Streptococcus sanguinis* (ATCC) was evaluated using the maceration method. The research was conducted at the Phytochemistry Laboratory and Microbiology Laboratory Faculty of Pharmacy, Universitas Sumatera Utara from march to may 2025. The study population consisted of *Streptococcus sanguinis* obtained from bacterial culture stocks at the Microbiology Laboratory, Faculty of Pharmacy, Universitas Sumatera Utara, and black cumin from Pusat Pasar, Medan.

Primary data in this study consisted of inhibition zone diameters of black cumin extract against *Streptococcus sanguinis* (ATCC). Black cumin were ground using a blender and sieved to obtain a fine powder. A total of 500 gram of the powder was placed into 5 liter jar and macerated with 96% ethanol until fully submerged. The jar was sealed with aluminium foil and left for 24 hours with stirring every 30 minutes during the first 6 hours. The jar was stored away from direct sunlight. The mixture was filtered using whatman filter paper and the residue was remacerated for 48 hours with 96% ethanol. The filtrates were combined and concentrated using a rotary evaporator at 60°C to obtain the crude extract. Extract solutions were prepared at concentrations of 25% (0.25 ml extract with 0.75 ml DMSO), 50% (0.5 ml extract with 0.5 ml DMSO), and 75% (0.75 ml extract with 0.25 ml DMSO).

The antibacterial activity using Kirby Bauer disc diffusion method. The study consisted of three groups such as black cumin extract at 25%, 50%, and 75% concentrations in group I, negative control (DMSO) in group II, positive control (chlorhexidine) in group III. Each test was performed in triplicate. Sterile blank discs were impregnated with the respective extract concentrations, chlorhexidine (positive control) or DMSO (negative control) and allowed to dry. Each Petri dish contained three discs with the extract concentrations and two discs with the control agents. Plates were incubated at 37°C for 24 hours, after which inhibition zones were measured in millimeters. The data was analyzed statistically using One Way ANOVA and Post Hoc (Tukey) test.

RESULTS AND DISCUSSION

This study utilized samples of black cumin (*Nigella sativa*) to test the antibacterial activity against *Streptococcus sanguinis*. The ethanol extract was obtained through maceration of 800 gram black cumin with 96% ethanol and producing 6 L of greyish filtrate. Evaporation using a rotary evaporator yielded 250.39 gram of dark extract which was subsequently used for antibacterial testing. Phytochemical testing was also conducted to identify secondary metabolites in black cumin as shown in the table below.

Table 1. Phytochemical Test Results of Black Cumin (*Nigella sativa*) Ethanol Extract

Phytochemical Result	Screening Result (+/-)
Flavonoid	+
Saponin	+
Alkaloid	+
Tannin	+
Steroid	+
Phenol	+

According to table 1 that black cumin extract positively contains metabolite compunds such as flavonoid, saponin, alkaloid, tannin, steroid, and phenol.

Table 2. Average Diameter of Inhibition Zone of Black Cumin (*Nigella sativa*) Extract against Streptococcus sanguinis Bacteria

Inhibition Zone (mm)	Black Cumin Extract Activity Test Results			
	Repitition 1	Repitition 2	Repitition 3	Ave

25% Concentration	11.2	10.8	9.9	10.63
50% Concentration	11.3	10.7	10.0	10.66
75% Concentration	10.9	11.7	10.1	10.9
Positive Control	13.8	11.6	12.4	12.6
Negative Control	0	0	0	0

Table 2 indicates that the inhibition zone of black cumin extract against *Streptococcus sanguinis* falls into the moderate category at all concentrations, with average diameters of 10.63 mm (25%), 10.66 mm (50%), and 10.9 mm (75%). The positive control sample exhibited a strong inhibition zone of 12.6 mm, while the negative control using DMSO showed no observable bacterial inhibition zone.

Table 3. Analyze The Inhibition Zone Diameter Using ANOVA

	Sum of Squares	df	Mean Square	F	Sig
Between Groups	.011	3	.004	3.717	.061
Within Groups	.008	8	.001		
Total	.019	11			

Based on the table above, a significant difference can be observed between the test groups in inhibiting the growth of *Streptococcus sanguinis*, with a p-value of 0.061 ($p < 0.1$).

Table 4. Comparison Test of Inhibition Zone Diameters With Post Hoc (Tukey)

(I) Experimental Group	(J) Experimental Group	Mean Difference (I-J)	std. error	Sig	90% Confidence Interval	
					Lower Bound	Upper Bound
Positive Group	EEBC 25% Group	.07316	.02560	.081	.0037	.1426
	EEBC 50% Group	.07177	.02560	.088	.0024	.1412
	EEBC 75% Group	.06261	.02560	.145	-.0068	.1320
EEBC 25% Group	Positive Group	-.07316	.02560	.081	-.1426	-.0037
	EEBC 50% Group	-.00140	.02560	1.000	-.0708	.0680
	EEBC 75% Group	-.01055	.02560	.975	-.0800	.0589
EEBC 50% Group	Positive Group	-.07177	.02560	.088	-.1412	-.0024
	EEBC 25% Group	.00140	.02560	1.000	-.0680	.0708
	EEBC 75% Group	-.00916	.02560	.983	-.0786	.0603
EEBC 75% Group	Positive Group	-.06261	.02560	.145	-.1320	.0068
	EEBC 25% Group	.01055	.02560	.975	-.0589	.0800
	EEBC 50% Group	.00916	.02560	.983	-.0603	.0786

The Post Hoc (Tukey) test showed a significant difference ($p < 0.1$) between positive control (chlorhexidine) and black cumin extract at concentrations of 25% ($p = 0.081$) and 50% ($p = 0.088$), indicating that the positive control had a stronger antibacterial effect. However, there was no significant difference between chlorhexidine and the 75% extract ($p = 0.145$), suggesting that the 75% concentration had a comparable inhibitory potential against *Streptococcus sanguinis*.

Phytochemical screening of black cumin (*Nigella sativa*) extract was found to be positive for the presence of active compunds, such as flavonoids, saponins, alkaloids, tannins, steroids, and phenols (Table 4.1). This finding is also supported by the research of Zuraida *et al*. (2022), identified secondary metabolites in black cumin extract with antimicrobial activity, including alkaloids, flavonoids, and saponins. Alkaloids are among the largest and most diverse groups of active compounds found in plants. They have diverse chemical structures and contain nitrogen atoms in heterocyclic rings. Alkaloids such as cicleanine and coxsacklin have been reported to exhibit antibacterial and antifungal activities (Sireesha *et al*., 2019).

The most abundant phenolic compound found in the ethanolic extract of black cumin (*Nigella sativa*) is flavonoids. Flavonoids act by increasing cell membrane permeability, disrupting the bacterial cell membrane structure, and causing the leakage of essential molecules and ions from within the cell. In addition, flavonoids also inhibit bacterial enzymatic activity and cellular respiration processes. *Nigella sativa* exhibits antibiofilm activity and is capable of inhibiting the growth of cariogenic bacteria, including *Streptococcus sanguinis* (Kurnia *et al*., 2024). The active flavonoid compounds in black cumin possess antibacterial, antioxidant, and immunomodulatory properties (Supriyana *et al*., 2019).

The antibacterial activity of black cumin extract (*Nigella sativa*) against *S. sanguinis*, as shown in table 4.2 demonstrated an average inhibition zone diameter categorized as moderate at all concentrations, with values of 10.63 mm (25%), 10.66 mm (50%), and 10.9 mm (75%). Meanwhile, the negative control using DMSO did not produce any bacterial inhibition zone, whereas the postive control exhibited an inhibition zone of 12.6 mm, which falls into the moderate category. These findings are consistent with the study by Ernawati *et al*., (2023), which reported that concentrations of 50% (12.6 mm), 75% (15.8 mm), and 100% (17 mm) resulted in average inhibition zone diameters classified as strong. This finding aligns with research by Sutrisna *et al*. (2022) observed inhibition zones of ethanolic black cumin extract against *Staphylococcus aureus* at concentrations of 12.5% (11.06 mm), 25% (29.58

mm), 50% (28.22 mm), and 100% (30.84 mm), while the positive control produced an inhibition zone of 24.31 mm.

The statistically analysis of the antibacterial activity of black cumin extract against *S. sanguinis* at concentrations of 25%, 50%, and 75% began with normality and homogeneity testing using the Saphiro Wilk method. The normality test results indicated that the extract at all three concentrations was normally distributed as evidenced by the significance value of 0.448 ($p > 0.05$), suggesting that the inhibition zones of *S. sanguinis* met the assumption of normality. Therefore, to examine the differences in mean inhibition zones among the extract concentrations, a parametric Analysis of Variance (ANOVA) test was performed.

The analysis of inhibition zone diameters using ANOVA yielded a p-value of 0.061 ($p < 0.1$), as presented in Table 4.3. This result indicates that there were significant differences among the test groups in inhibiting the growth of *S. sanguinis*, demonstrating that the three concentrations of black cumin extract produced significantly different bacterial inhibition zones. Further pairwise comparisons between concentrations were conducted using the Post Hoc Tukey Test.

The pairwise comparison of black cumin extract concentrations using the Post Hoc Tukey test, as presented in Table 4.4, revealed that the positive control (chlorhexidine) exhibited a significant difference ($p < 0.1$) in inhibiting *S. sanguinis* compared to the 25% extract group ($p = 0.081$) and the 50% extract group ($p = 0.088$). This indicates that chlorhexidine has a stronger antibacterial effect than black cumin extract at concentrations of 25%, and 50%. In contrast, no significant difference was observed between the 75% extract group and the positive control ($p > 0.1$), suggesting that the 75% extract has a comparable potential to chlorhexidine in inhibiting the growth of *Streptococcus sanguinis*.

Previous studies, such as Ernawati *et al*. (2024) demonstrated that the black cumin extract effectively inhibited *S. aureus*, with the strongest inhibition observed at 100% concentration. Similarly, research by Salsabila *et al*. (2025) found that black cumin oil exhibited stronger activity against gram positive bacteria (*S. aureus*) compared to gram negative (*Pseudomonas aeruginosa*). While, Haryati *et al*. (2020) reported the antibacterial effect of toothpaste containing black cumin seed extract against *Lactobacillus acidophilus* with the highest activity at 15%.

CONCLUSIONS

The black cumin (*Nigella sativa*) extract inhibited *Streptococcus sanguinis* at a concentration of 75% (10.9 mm) with a significant value of 0.145 (p>0.1), which is categorized as a moderate inhibition zone. Antibacterial activity testing can be extended to other bacterial species, both gram positive and gram negative in order to determine the broad spectrum activity of the extract.

ACKNOWLEDGEMENT

Thank you to all parties who have helped make this research possible, including the dental health and pharmacy lecturers and laboratory technicians at the Faculty of Pharmacy, University of North Sumatra.

REFERENCES

Asridiana, A., & Thioritz, E. (2020). Efektivitas Penggunaan Obat Kumur Beralkohol Dan Non-Alkohol Terhadap Penurunan Indeks Plak Mahasiswa D-Iv Jurusan Keperawatan Gigi Poltekkes Makassar. *Media Kesehatan Gigi : Politeknik Kesehatan Makassar*, *18*(2), 1–8. https://doi.org/10.32382/mkg.v18i2.1306

Ernawati, K., Yudistian, I., & Putri, N. (2024). *Uji Daya Hambat Ekstrak Jintan Hitam (Nigella sativa) Konsentrasi 50 %, 75 % Dan 100 % Terhadap Pertumbuhan Bakteri Staphyloccocus aureus*. 643–654. https://e-journal.unmas.ac.id/index.php/BDSE/article/download/10826/8142

Ernawati, L., Sumantri, S., Wedagama, W., Astuti, A., & Fatmasari, D. (2023). Effectiveness of Black Cumin Extract (*Nigella Sativa*) in Inhibiting The Growth of *Staphylococcus Aureus* Bacteria in Vitro. *Jurnal Kesehatan Gigi*, *10*(1), 56–63. https://doi.org/10.31983/jkg.v10i1.9531

Haryati, E., Wahyu, R., Haryati dan, E., Wahyu Pratiwi, R. (2020). Uji Aktivitas Antibakteri Pasta Gigi Ekstrak Biji Jintan Hitam (*Nigella sativa L*) Terhadap Bakteri *Lactobacillus achidophilus*. *Jurnal.Stfypibcirebon.Ac.Id*, *4*(1), 2020–2021. http://jurnal.stfypibcirebon.ac.id/index.php/ojs/article/view/71

Indonesian Ministry Of Health Development Policy Board. (2023). Indonesian Health Survey (Survei Kesehatan Indonesia) 2023. *Ministry of Health*, 1–68.

Kurnia, D., Padilah, R., Apriyanti, E., & Dharsono, H. (2024). Phytochemical Analysis and Anti-Biofilm Potential That Cause Dental Caries from Black Cumin Seeds Phytochemical Analysis and Anti-Biofilm Potential That Cause Dental Caries from Black Cumin Seeds *(Nigella sativa Linn .)*. https://doi.org/10.2147/DDDT.S454217

Kasuma N. (2016). *Plak Gigi*. [e-book] Andalas University Press. Available at : http://repo.unand.ac.id/29800/1/PLAK%20GIGI.pdf

Makmun, A., Surdam, Z., & Gunawan, A. M. (2020). Uji Efektivitas Ekstrak Jintan Hitam (*Nigella Sativa*) Terhadap Pertumbuhan Bakteri *Staphylococcus Aureus* Pada Medium

MHA (*Mueller Hinton Agar*). *Window of Health : Jurnal Kesehatan*, *3*(1), 1–9. https://doi.org/10.33368/woh.v0i0.204

Prasko, P., Widyatmoko, Y., & Sadimin, S. (2022). The Effect of Gargling Areca Nut Extract (*Areca catechu L*) 2,5% Against Plaque pH and Saliva pH. *Jurnal Kesehatan Gigi*, *9*(2), 148–153. https://doi.org/10.31983/jkg.v9i2.8504

Ristianti, N., W, J. K., & Marsono. (2015). Perbedaan Efektifitas Obat Kumur Herbal dan Non Herbal Terhadap Akumulasi Plak di Dalam Rongga Mulut. *Medali Jurnal*, *2*(1), 31–36. https://doi.org/10.30659/medali.v2i1.447

Salsabila, M., Rukaya, B., & Syuhada. (2025). Evaluasi Aktivitas Antibakteri Minyak Jintan Hitam terhadap Bakteri Gram-Positif dan Gram-Negatif : Studi pada *Staphylococcus aureus dan Pseudomonas aeruginosa*. *13*(1), 218–226. https://doi.org/10.33394/bioscientist.v13i1.14950

Sari, D. P., Aspriyanto, D., & Taufiqurrahman, I. (2020). Antibacterial Effectivity Of Kasturi Leaf Extract (*Mangifera Casturi*) Against The Growth Of *Streptococcus Sanguinis* Bacteria. *Dentino : Jurnal Kedokteran Gigi*, *5*(1), 33. https://doi.org/10.20527/dentino.v5i1.8118

Satrio, G., Chusna, N., Priyadi, M.(2020). Uji Potensi Daya Hambat Ekstrak Metanol Biji Jintan Hitam (*Nigella Sativa L.*) Terhadap Bakteri *Streptococcus*. *Jurnal Surya Medika*, *6*(1), 18–21. http://journal.umpalangkaraya.ac.id/index.php/jsm

Sireesha, B., Reddy, B. V., Basha, S. K., Chandra, K., & Anasuya, D. (2019). a Review on Pharmacological Activities of Alkaloids. *World Journal of Current Medical and Pharmaceutical Research*, *01*(06), 230–234. https://doi.org/10.37022/wjcmpr.2019.01068

Sumadewi, K. T., & Harkitasari, S. (2023). Edukasi kesehatan gigi dan mulut serta cara menggosok gigi pada anak sekolah dasar di Banjar Bukian, Desa Pelaga. *Journal WMMJ Warmadewa Minesterium Medical Journal*, *2*(1), 1–7. https://www.ejournal.warmadewa.ac.id/index.php/wmmj/article/view/6162

Supriyana, S., Aryati, E., Sadimin, S., & Utami, W. J. D. (2019). Kemampuan Obat Kumur Ekstrak Jinten Hitam Sediaan Kantong Celup Terhadap Monosit Dan Neutrofil Pada Adhesi *Streptococcus mutan*. *Link*, *15*(2), 36–41. https://doi.org/10.31983/link.v15i2.5384

Sutrisna, E., Wahyuni, S., & Fitriani, A. (2022). Antibacterial Effect of *Nigella sativa L*. Seed from Indonesia. *Pharmacognosy Journal*, *14*(6), 1029–1032. https://doi.org/10.5530/pj.2022.14.206

Syahrani, N., Kurniawati, A., Prihanti, A. M., Sulistyani, E., & Lestari, P. E. (2024). Uji Aktivitas Antibakteri Ekstrak Etanol Daun Ungu (*Graptophyllum pictum (L) Griff*) terhadap Pertumbuhan *Streptococcus sanguinis*. *Pustaka Kesehatan*, *12*(1), 19. https://doi.org/10.19184/pk.v12i1.42325

Waty, S., Lusiani, Y., & Hidayah, N. 2023. Cegah Karies Gigi Dengan Ekstrak Kulit Kayu Manis. Yogyakarta : *Deepublish Digital*

Zakki, M. (2017). Uji Aktivitas Antibakteri Ekstrak Cathechin Teh Putih Terhadap *Streptococcus sanguinis*. *ODONTO : Dental Journal*, *4*(2), 108. https://doi.org/10.30659/odj.4.2.108-113

Zhu, B., Macleod, L., Kitten, T., & Xu, P. (2018). *Streptococcus sanguinis* biofilm formation & interaction with oral pathogens. *Future Microbiology*, *13*(8), 915–932. http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L622901472%0Ahttp://dx.doi.org/10.2217/fmb-2018-0043

Zuraida, Z., Masdianto, M., & Jannah, H. Z. (2022). Uji Daya Hambat Ekstrak Jintan Hitam (*Nigella Sativa*) Terhadap Bakteri *Staphylococcus aureus* ATCC 25923. *Anakes : Jurnal Ilmiah Analis Kesehatan*, *8*(1), 109–118. https://doi.org/10.37012/anakes.v8i1.872