

Phytochemical Analysis and Anti-Biofilm Potential That Cause Dental Caries from Black Cumin Seeds (*Nigella sativa* Linn.)

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Abstract: The oral cavity is an excellent place for various microorganisms to grow. *Spectrococcus mutans* and *Spectrococcus sanguinis* are Gram-negative bacteria found in the oral cavity as pioneer biofilm formers on the tooth surface that cause caries. Caries treatment has been done with antibiotics and therapeutics, but the resistance level of *S. mutans* and *S. sanguinis* bacteria necessitates the exploration of new drug compounds. Black cumin (*Nigella sativa* Linn.) is known to contain secondary metabolites that have antioxidant, antibacterial, anti-biofilm, anti-inflammatory and antifungal activities. The purpose of this review article is to present data on the potential of *Nigella sativa* Linn seeds as anti-biofilm. This article will discuss biofilm-forming bacteria, the resistance mechanism of antibiotics, the bioactivity of *N. sativa* extracts and seed isolates together with the Structure Activity Relationship (SAR) review of *N. sativa* compound isolates. We collected data from reliable references that will illustrate the potential of *N. sativa* seeds as anti-biofilm drug.

Keywords: *Nigella sativa* Linn, dental caries, antibiotics, anti-biofilm, *S. mutans*, *S. sanguinis*

Introduction

The oral cavity is an excellent place for various microorganisms to grow, due to conditions that are always moist by saliva, especially on the surface of the teeth and mucosa. It is estimated that there are 500–1000 species of bacteria and fungi growing in the oral cavity.^{1,2} *Spectrococcus mutans* and *Spectrococcus sanguinis* are known to infect the oral cavity by synthesizing biofilm layer on the tooth surface that forms caries. As a biofilm-forming pioneer, *S. mutans* signals to other bacteria to form more colonies and strengthen the biofilm. *S. sanguinis* as one of the bacteria receiving signals from *S. mutans* cooperates to create a stronger biofilm layer. With the help of the enzyme gluconastransferase, the biofilm layer formed becomes caries.^{3,4} Dental caries is considered a chronic disease that can affect all groups. In fact, the handling and treatment of caries is still very lacking, which causes this disease to continue.⁵ In addition, the use of antibiotics as a cure for dental caries is resistant. Treatment is very urgent and new drug alternatives are needed. *Nigella sativa* Linn. seeds are known to contain bioactive compounds that have potential as an alternative treatment for dental caries.

N. sativa seed extract contains secondary metabolite compounds of terpenoids, alkaloids, flavonoids and phenolics.^{6,7} Several researchers have reported that the compounds contained in *N. sativa* seeds have antibacterial, anti-biofilm, anti-inflammatory and antifungal activities.^{8–10} Bioactivity of *N. sativa* has potential as a natural caries drug.¹¹ Based on these data, this review will focus on the activity of compounds from *N. sativa* seeds against biofilm-forming bacteria. This review will also review the Structural Relationship of compounds from *N. sativa* seeds and the mechanism of inhibition of biofilm-causing bacteria.

Biofilm Forming Gram-Positive and Gram-Negative Bacteria

Gram-Positive Bacteria

Spectrococcus mutans

S. mutans is an anaerobic Gram-positive bacterium found in the oral cavity.¹² The condition of the oral cavity always changes in pH, temperature and pressure requiring *S. mutans* adaptation. This situation causes *S. mutans* to synthesize new metabolites that change its physiological properties. The resulting consequences such as increased biofilm homeostasis become dental caries.¹³ *S. mutans* mediates gluconantransferase (Gtf), which forms extracellular polysaccharides (EPS) on the tooth surface. EPS is formed at the adhesion stage utilizing sucrose with the help of the enzyme gluconantransferase (Gtfs). This enzyme catalyzes the breakdown of sucrose into glucose and fructose. Through glycoside bonds extracellular glucans are formed (EPS). At this stage, the biofilm that forms is still small, followed by the proliferation stage, specific caries bacteria will survive on the biofilm so that caries forms.¹⁴ The EPS formed becomes a food source and ensures the survival of biofilm-forming bacterial colonies.^{14,15} In addition, EPS protects colonies from host attacks and antibiotics. Microorganisms such as *Candida albicans*, *Porphyromonas*, *Prevotella*, *Fusobacterium nucleatum* and others are incorporated into colonies contained in biofilms.¹⁶

Biofilms can be inhibited by stopping the synthesis of EPS catalyzed by Gtf enzymes (shown in Figure 1). This can be done using micro molecules, natural products, probiotics and prebiotics. The introduction of components into EPS results in changes in EPS regulation.¹⁷ At this stage, new EPS components are formed that need to be prevented from being occupied by new bacterial colonies using antibiotics. EPS degradation and the presence of antibiotics reduce biofilms and dental caries.¹⁸

In the biofilm formed by *S. mutans*, amyloid fibers of different sizes were found. Amyloid fibers are fibers that can protect the biofilm layer from aggregation and environmental influences such as enzymes. *S. mutans* synthesizes amyloid with the help of the enzyme transpeptidase sortase which connects the substrate and cell wall peptidoglycan. The characterization results of amyloid fibers formed have two different types of sizes and add to the biofilm inhibition route. The presence of molecules that can lyse amyloid fibers can reduce the attachment strength of biofilms.^{19,20}

Streptococcus sanguinis

Streptococcus sanguinis (*S. sanguinis*) is a class of Gram-positive mythical *Streptococcus* found in toddlers, adolescents and adults.²¹ Like its main pioneer (*S. mutans*), it is involved in forming biofilms that synergize with other bacterial species such as *Corynebacterium durum*.²² Multiple biofilms were observed in OKF4/TERT-1 and hTERT TIGs artificial cells containing *S. sanguinis* and *C. durum* as commensal bacteria and *Porphyromonas gingivalis* as pathobionts. *S. sanguinis* contributed almost 96.6% to the multiple biofilms formed by the three species, while *C. durum* and *P. gingivalis* only 1.6 and 1.8%, respectively. A very dominant contribution was shown by *S. sanguinis* in biofilm

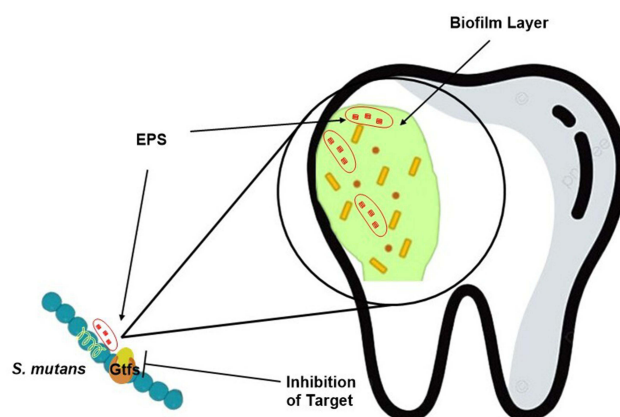


Figure 1 The mechanism of biofilm inhibition is by inhibiting the gluconantransferase enzyme of *S. mutans* as a catalyst for EPS formation. Unavailable EPS causes bacteria not to colonize to form biofilms.¹⁸

formation.²³ *S. sanguinis* also has the ability to ferment polysaccharides into acidic compounds. This causes demineralization of enamel in the teeth which initiates caries.²⁴

The close relationship between *S. mutans* and *S. sanguinis* affects the biofilm formed. Both support each other providing a source of attachment for other microorganisms.²⁵ However, research conducted by Cui et al membrane vesicles from *S. mutans* can inhibit biofilm formation from *S. sanguinis*. This unique property causes biofilm inhibition to be more diverse.²⁶

In addition to biofilm formation, *S. sanguinis* encodes pili IV in the metabolism that causes infective endocarditis (IE) disease. *S. sanguinis* can bind to the platelet protein matrix provided by *S. mutans* on biofilms.²⁷ This results in *S. sanguinis* being able to exit the oral cavity and be carried by the bloodstream to the heart. The accumulation of *S. sanguinis* in the heart initiates IE.²⁸

Gram-Negative Bacteria

Aggregatibacter actinomycetemcomitans

Aggregatibacter actinomycetemcomitans (*A. actinomycetemcomitans*) is a Gram-negative bacterium in the oral cavity.²⁹ *A. Actinomycetemcomitans* is incorporated into biofilm colonies that affect the rate of biofilm formation. Although not very significant, the gene expression of *A. Actinomycetemcomitans* in biofilm is most dominant compared to other Gram-negative bacteria (*P. Gingivalis*, *V. dispar*, *A. oris*, and *S. anginosus*).³⁰

A. Actinomycetemcomitans expresses a fimbriae protein and three non-fimbriae surface proteins (EmaA, Aae and ApiA). These proteins play a role in forming biofilms on the tooth surface. EmaA protein expression is known to be the most dominant in biofilm formation compared to other non-fimbriae proteins.³¹ OmpA1 and OmpA2 are also expressed which ensures the survival of *A. Actinomycetemcomitans*. OmpA1 and OmpA2 can interact with C4 binding pockets that inhibit lectin activity. This binding ability is what makes *A. Actinomycetemcomitans* resistant.³²

Antibiotics

Antibiotics are compound species that can inhibit and/or kill a pathogenic microorganism. In the early era, the discovery of antibiotics gave many changes to the world of medicine.³³ There was an extension of human lifetime compared to before the discovery of antibiotics.³⁴ A very big discovery turned out to have a big negative impact. In addition to high resistance to antibiotics, massive use has an impact on environmental pollution. Exposure to waste such as in fresh water is difficult to degrade and threatens survival.³⁵

Common Antibiotics Used in Caries Treatment

Rifamycin

Rifamycin is an antibiotic that has an aromatic ring structure (shown in Figure 2). Excellent activity is shown by rifamycin against Gram-positive and Gram-negative bacteria. The mechanism of rifamycin inhibition is through inhibiting the enzyme RNA polymerase (RNAP) which causes RNA synthesis to be inhibited. The structure of rifamycin has hydroquinone which can be auto-oxidized by the enzyme Rox monooxygenase in bacterial cells. Biofilm forming bacteria such as *Staphylococcus aureus* have the ability to repair genetic information systems. This causes rifamycin to be unable to inhibit RNA synthesis from *S. aureus*, making it resistant.^{36–38}

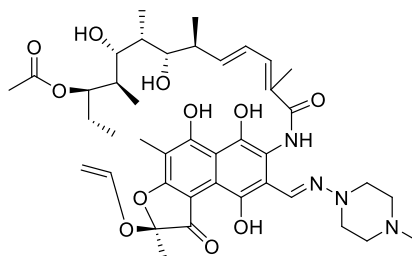


Figure 2 Structure of Rifamycin.³⁷

Amoxicillin

Amoxicillin has been used as an antibiotic since 1970 with a chemical structure having a β -lactam ring (shown in Figure 3). Treatment of dental caries infected by Gram-positive and Gram-negative bacteria often uses amoxicillin.³⁹ The inhibitory mechanism of amoxicillin by damaging the bacterial cell wall. Research results show that the use of amoxicillin increases the ability of bacteria to express β -lactamase enzymes that have structures similar to amoxicillin. Expression of β -lactamase enzyme makes bacteria recognize amoxicillin in the next treatment which causes resistance.³⁶ *S. sanguinis* and *S. mitis* are caries-causing bacteria that are resistant to amoxicillin.⁴⁰

Chlorhexidine

Chlorhexidine is often used to treat dental caries to date. In the application of chlorhexidine, it is able to change the pH conditions in the oral cavity characterized by differences in the concentration of lactic acid, nitrate and nitrite.⁴² The attachment of chlorhexidine to the bacterial cell wall is through charged interaction. Bacterial cell walls are negatively charged, while chlorhexidine ions are positively charged so that attachment occurs. Changes in pH in the oral environment cause an imbalance to occur, increasing the ability of microorganisms to express certain proteins. Like the main bacteria that cause dental caries, *S. mutans* is able to express the *dlt* operon which changes the surface structure of the cell wall to be very hydrophobic. As a result, the cell wall is positively charged and no ionic interaction with chlorhexidine occurs. The expression of the *dlt* operon from *S. mutans* causes it to be resistant to chlorhexidine (shown in Figure 4).⁴³

Florida Toothpaste

Florida is often used in toothpaste because of its excellent antibiotic activity. Florida can prevent tooth decay caused by pathogenic microorganisms. The tooth surface layer is re-mineralized by fluoride ions resulting in the erosion of dental calcium and phosphate to form the compound fluoride apatite $[\text{Ca}_5(\text{PO}_4)_3\text{F}]$. Florida apatite is very useful for protecting teeth and preventing biofilm formation. In addition, the presence of chloride can suppress the amount of acid produced by bacteria so that teeth are protected from caries.^{44,45}

The element fluoride has the highest electronegativity value among other chemical elements. Florida can attract electrons from bone and tooth building blocks such as calcium and cause damage to multicellular cell organelles. The use

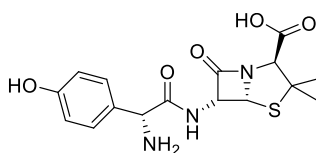


Figure 3 β -lactam structure of amoxicillin.⁴¹

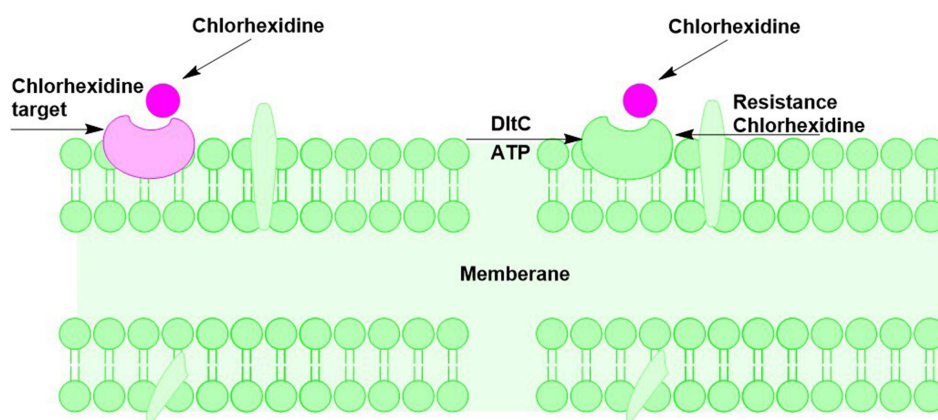


Figure 4 Bacterial member as a target for chlorhexidine attachment and bacterial member that is resistant to chlorhexidine due to the differential charge on the member surface by DltA.⁴³

of fluoride in toothpaste in low concentrations is very beneficial, but the accumulation of fluoride due to continuous use raises concerns.⁴⁵ According to Tang et al, 2019 fluoride has a level of resistance to the cas3 gene that is expressed by the biofilm-forming bacteria *Streptococcus mutans* modifications to fluoride are needed to target cas3 so as to reduce resistance.⁴⁶

Antibiotics as MurA Enzyme Inhibitors

Various antibiotics target the destruction of the bacterial cell wall that holds the cell unit together. The main component of the bacterial cell wall is peptidoglycan, which is composed of a layer of disaccharides crosslinked with amino acids through peptide bonds.⁴⁷ Peptidoglycan is synthesized by a series of biological mechanisms in the cytoplasm, member and periplasm catalyzed by Mur (Muramidase) enzymes.⁴⁸ As well as caries-causing bacteria *S. mutans* synthesize peptidoglycan with the help of the enzyme Mur.⁴⁹ The enzymes MurA and MurB transfer PEP (phosphoenolpyruvate) which converts UDP-GlcNac (uridine diphosphate-*N*-acetylglucosamine) into (uridine diphosphate-*N*-acetylmuramyl-pentapeptide) UDP-MurNAc.⁵⁰ UDP-MurNAc is then called the substrate to which the amino acids L-ala, D-Glu, L-Lys and D-Ala-D-Ala will be attached with the help of the enzymes MurC, MurD, MurE and MurF (shown in Figure 5). The amino acids on the substrate crosslink with the disaccharide to form a peptidoglycan. The enzyme MurA has been recognized as an important factor in peptidoglycan synthesis and is a target in antibiotic discovery.⁵¹

Secondary Metabolites as Caries Antibacterials

Flavonoids

Flavonoids are a class of secondary metabolites that have three distinctive rings. Flavonoid structures are classified into flavanone, flavanone, isoflavone, dihydroflavanol, flavonols, flavan-3,4-diol, flavan-3-ol and anthocyanins.⁵² The fruitful structure makes flavonoids have antibacterial activity against Gram-positive and Gram-negative bacteria. The mechanism of flavonoid inhibition is through the interaction of hydroxy groups with bacterial proteins that are very important in metabolisms such as with topoisomerase, helicase, and DNA gyrase proteins.⁵³ This is based on the ability to transfer electrons and stabilize the core structure.^{17,52,54–56}

Terpenoids

Terpenoids have antibacterial activity against oral pathogens by damaging bacterial cell membranes.^{56–59} Damaged cell membranes evidenced by protein release from oral bacterial cell membranes.⁶⁰

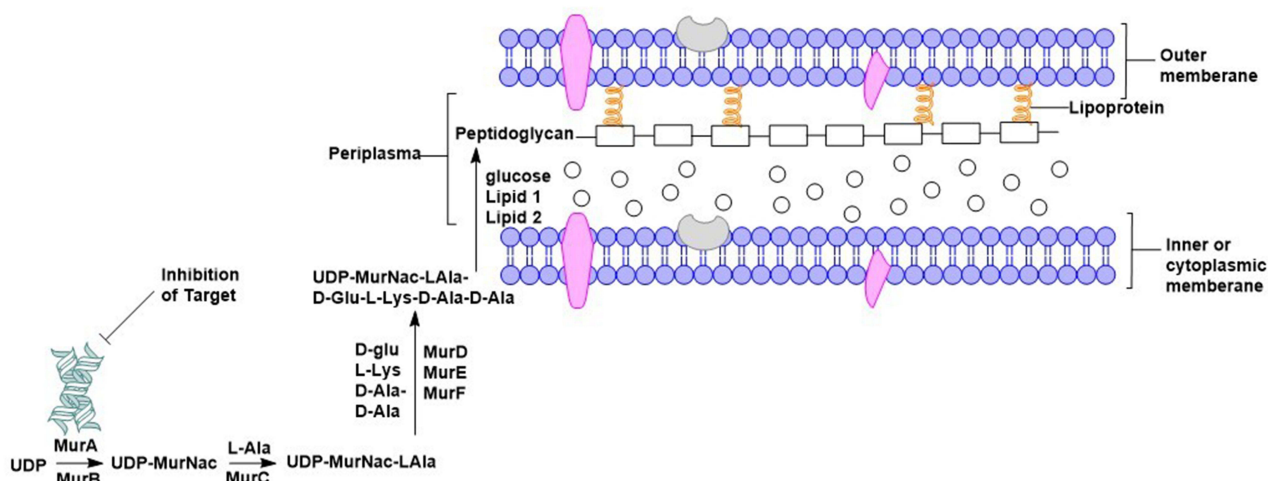


Figure 5 Peptidoglycan formation mechanism. The MurA enzyme catalyzes the initial phase of peptidoglycan synthesis, making it a potential target for antibiotics to prevent bacterial cell wall formation.⁴⁷

Alkaloids

Alkaloids inhibit the formation of peptidoglycan which causes damage to the bacterial cell wall. Alkaloids are known to have antibacterial activity against oral pathogens such as *S. mutans* and *P. gingivalis*.^{59–63}

Phenolic

Phenolic group compounds have antibacterial activity that causes dental caries.^{64,65} The antibacterial mechanism of the phenolic group by damaging the bacterial cell wall. The hydroxy group bound to the benzene ring allows the release of hydrogen atoms that will interact with the membrane wall and changes in membrane permeability occur.^{64–67}

Saponins

The saponin group has anti-inflammatory, anticancer and antibacterial therapeutic activities. As antibacterial saponins damage bacterial cells, causing cell death. Pathogenic oral bacteria such as *P. gingivalis* that cause caries and bad breath can be inhibited by this group.^{67–71}

Tannins

Tannins have diverse pharmacological activities due to their fertile structure. As oral antibacterial agents, tannins have the ability to inhibit quorum sensing and biofilm formation. Through the mechanism of inhibiting cell wall synthesis, member lysis and inhibiting the enzyme gluconastransferase, tannins can inhibit the formation of biofilms.^{72–74}

Black Cumin (*Nigella sativa* Linn.)

Black cumin (*Nigella sativa* Linn.) is a plant from the Ranunculaceae family that is widely cultivated in Asia, Europe, Africa and the Americas.⁷⁵ Native to the middle and far eastern regions, *N. sativa* seeds have abundant ethnopharmacology.^{76,77} Anti-inflammatory, antioxidant, cardiovascular, anticancer, antibacterial and antifungal are some of the known pharmacological activities of *N. sativa*.^{75,78} Black cumin seeds have been widely used traditionally to treat cancer, mental disorders and treat bacterial-induced diseases.⁷⁹ In various parts of the world, black cumin seeds have been consumed as a safe spice that has immune-boosting properties.⁸⁰ Ethnopharmacology is supported by secondary metabolite compounds contained in black cumin seeds. Alkaloids, polyphenols, phytosterols, terpenes, terpenoids and essential oils are secondary metabolites in *N. sativa*.⁸¹

Secondary Metabolites *Nigella sativa* Linn

Volatile Compound

Samples of *N. sativa* seeds from Bangladesh and India were analyzed for volatile compound components using GC-MS. A total of 200 g of black cumin seeds were ground and dried at 40 °C. The powder was analyzed using GC-MS A Hewlett-Packard (HP) 6890 Series II with FID detector and helium carrier gas. The analysis revealed volatile compounds octanoic acid, thymoquinone, thymol, *p*-cymene, maculosin, hygrine, ethyl ester and 2-monomyristin with main components thymoquinone, thymol and *p*-cymene are as shown in Figure 6.⁸² Volatile compounds from *N. sativa* seed extracts have oral antibacterial activity as on *S. aureus* and *P. aeruginosa*.^{79,83}

Polyphenols

Polyphenols were isolated from methanol and acetone extracts of *N. sativa* seeds. Separation using HPLC system variant pro-star model 230 with reversed-phase column. Eluents used were acetonitrile and glacial acetic acid with a 5% gradient. The methanol extract contained more polyphenol components than the acetone extract with concentrations of 13,714 and 0.5962 mg/g, respectively.⁸⁹ Polyphenolic compounds that were successfully isolated were reported by Enomoto et al was 2-(2-methoxy propyl)-5-methyl-1,4-benzenediol is shown in Figure 7.⁹⁰

Terpenoids

Terpenoids are a class of nonpolar compounds obtained from *N. sativa* L. seed oil are shown in Figure 8. Isolation of compounds can be done using preparative chromatography and open column chromatography. The oil from the refined seeds was extracted using the hydro-distillation method and columnized with the norm phase with the solvent *n*-hexanes-diethyl ether.⁹²

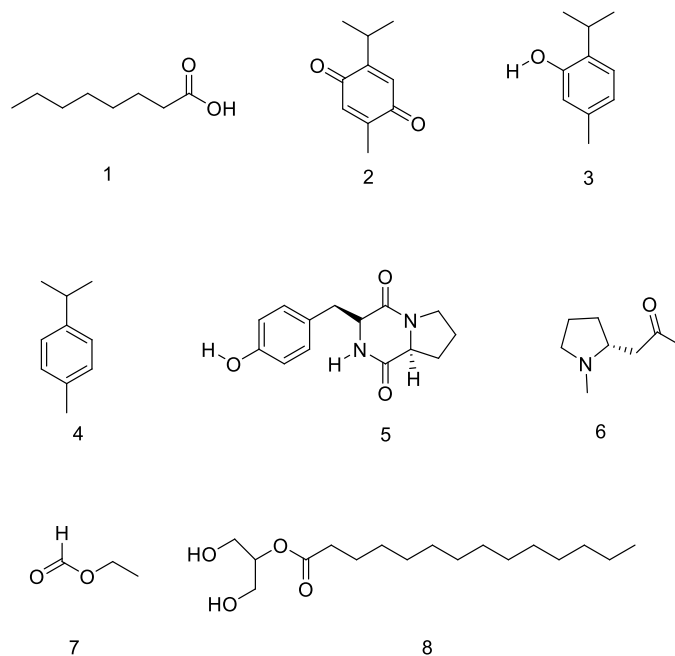


Figure 6 Major volatile compounds of *Nigella sativa* Linn. oil, octanoic acid (1), thymoquinone (2), thymol (3), *p*-cymene (4), maculosin (5), hygrine (6), ethyl ester (7), 2-monomyristin (8).^{6,82,84–88}

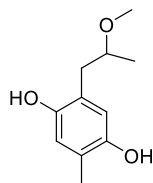


Figure 7 Structure polyphenols of 2-(2-methoxy propyl)-5-methyl-1,4-benzenediol.⁹⁰

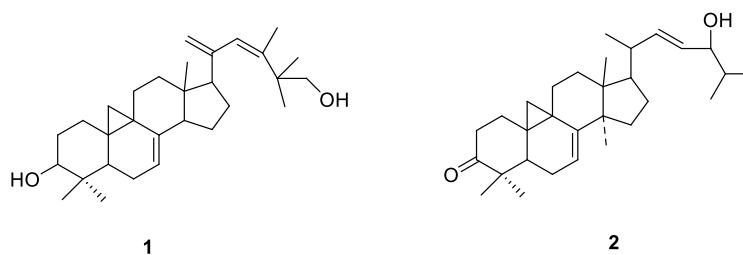


Figure 8 Terpenoid structures of *N. sativa* isolates cycloart-23-methyl-7,20,22-triene-3b, 30-diol (1) and cycloart-3-one-7,22-diene-24-ol (2).⁹⁶

Alkaloids

Alkaloids were obtained from the polar fraction of *N. sativa* seed extracted using methanol-water 3:1. The dried polar fraction was analyzed using UPLC-C8-FT-MS/MS. The polar fraction was dissolved in 2000 μ L of UPLC-grade MeOH/H₂O (1:1, v/v). The column used was a C18 stationary phase, using a Waters Acquity UPLC system. Mass spectra were used to analyze the polar components through fragmentation patterns via ESI (-) and ESI (+) ionization methods.⁹⁴ Some of the alkaloid compounds successfully isolated from *N. sativa* seed are shown in Figure 9.

Saponins

Saponins can be extracted from *N. sativa* seeds using polar solvents such as methanol, butanol and ethanol. Saponin phytochemistry is very distinctive and can be analyzed quantitatively using TLC. The R_f (Retentions factor) value of

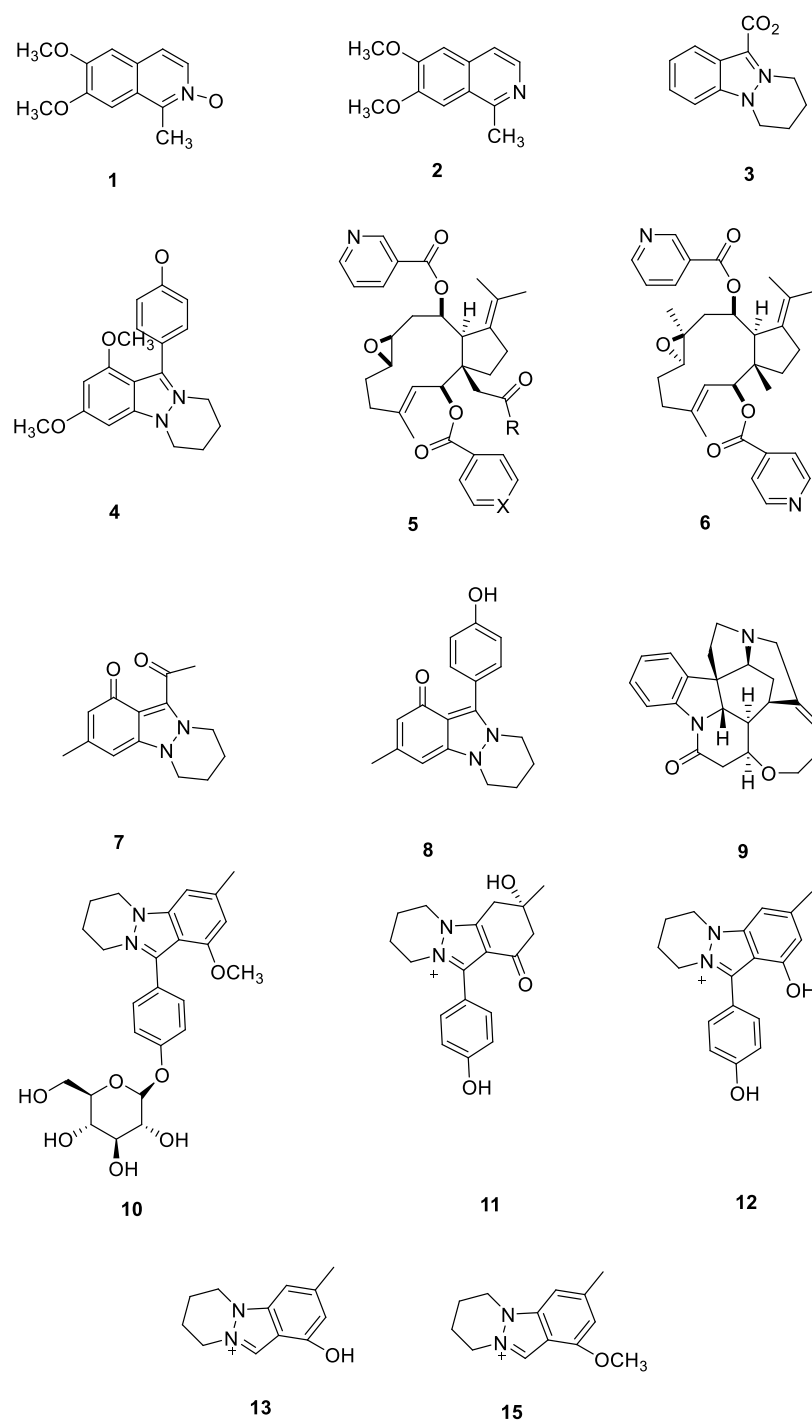


Figure 9 Alkaloid structures of *N. sativa* isolates Derivate isoquinolin (1), 6,7-dimethoxy-1-methylisoquinoline (2), Nigellidine (3), Nigellidine (4), Nigellamines ((A1: (5a) R = Ph, X = CH), (A2: (5b) R = Ph, X = (N), (A3: (5c) R = n-C₃H₇, X = (N), (A4: (5d) R = n-C₃H₇, X = (N), (A5: (5e) R = PhCH₂, X = (N)), Nigellamine C (6).^{91,92,95,96} Nigellidine (7).⁹³ Nigellidine (8).^{97,98} Strychnine (9).⁹⁹ 17-O-(β-D-glucopyranosyl)-4-O-methylnigellidine (10), nigelanoid (11), 7-O-(β-D-Glucopyranosyl)-4-O-methylnigellidine (12), nigellicine (13), 8b4-O-methylnigellidine, 8bnigeglanine (14) dan 4-O-methylnigeglanin (15).¹⁰³

saponins on the TLC plate is 0.8. TLC can be used in qualitative phytochemical analysis based on the R_f value.^{97,101,102} Isolation of saponins from butanol extract can be done by open column chromatography. From column chromatography, twelve saponin isolates were obtained and are shown in Figure 10.¹⁰⁰

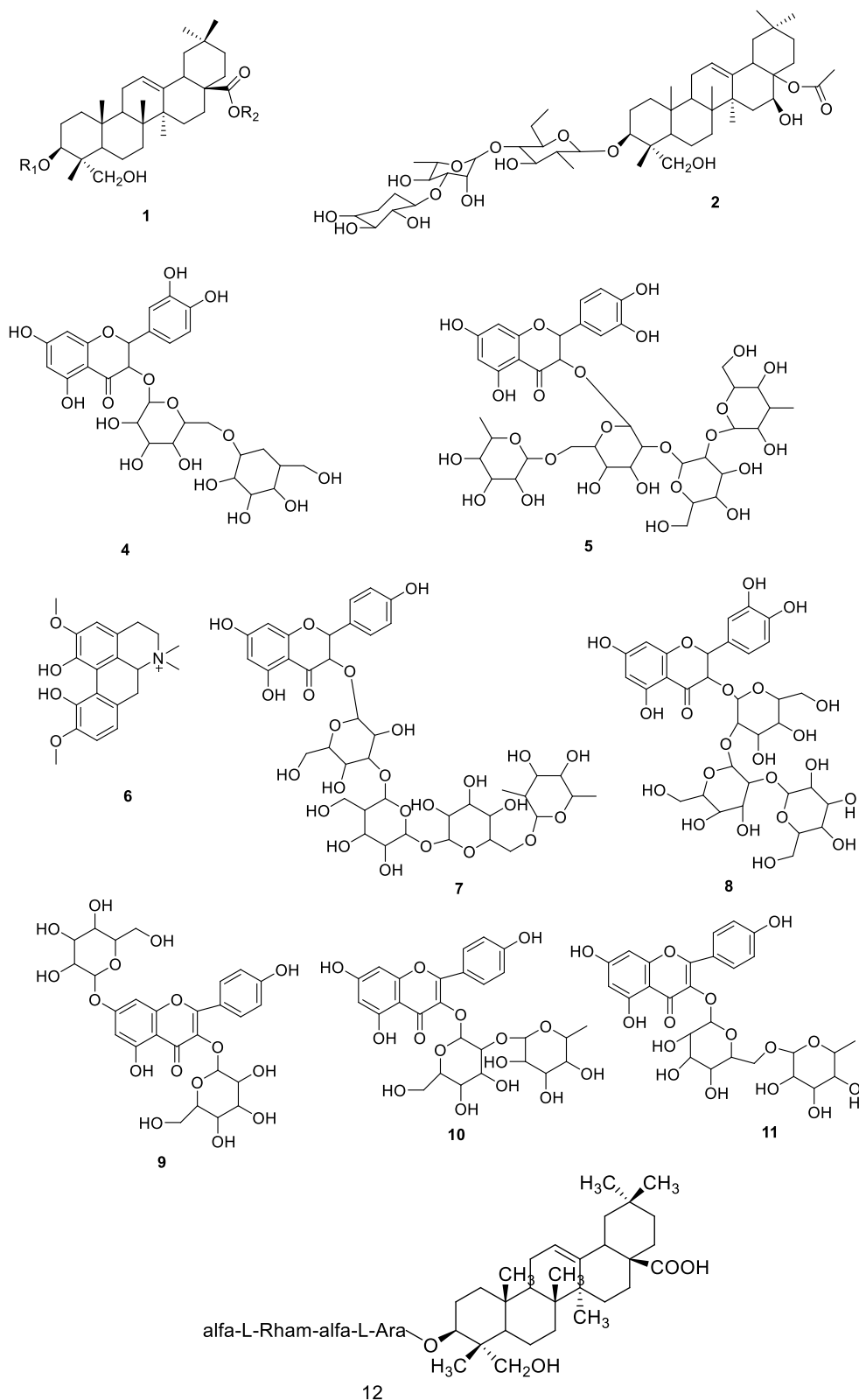


Figure 10 Saponins structure of *N. sativa* are R1 = β -D-Xylp-(1-3)- α -L-Rhap-(1-2)- α -L-Arap-(b. R2 = α -L-Rhap-(1-4)- β -D-Glcp-(1-6)- β -D-Glcp-(1)(1),^{91,103} 3-O-[β -D-xylopyranosyl-(1-3)- α -L-rhamnopyranosyl-(1-4)- β -D-glucopyranosyl]-11-methoxy-16-hydroxy-17-acetoxy (2),¹⁰⁴ a. R1 = β -D-xylp-(1-2)- β -D-glcp-(1-b. R2 = α -L-rhap-(1-4)- β -D-glcp-(1-6)- β -D-glcp-(1-flaaccidoside III (3), quercetin-3-gentiobioside (4), nigelflavonoid B (5), magnoflorine (6), nigelloside (7), quercetin sphorotrioside (8), kaempferol-3,7-diglucoside (9), kaempferol 3-O-rutinoside (10), rutin (11)¹⁰⁰ α -hederin (α -HN) (12).¹⁰⁹

Bioactivity of Extracts and Compounds of *Nigella sativa* Linn

Bioactivity of *Nigella sativa* Linn Extracts and Compounds

The content of compounds in extracts, oils and pure isolates from *N. sativa* seeds has been widely known and applied.¹⁰⁶ As in the Middle East, Europe and Asia, traditional and functional applications of *N. sativa* seeds are very promising drugs against various diseases.¹⁰⁷ Development of compounds in *N. sativa* seeds continues to be carried out to increase activity.¹⁰⁸ *N. sativa* bioactivities from different countries are presented in Table 1.

Antibacterial Activity of Oral Pathogens of *Nigella sativa* Linn

The antibacterial activity of *N. sativa* seeds against oral pathogenic bacteria is very good.¹²¹ Especially the bacteria that cause caries extract and compound showed very positive activity.^{105,110,111,122} *N. sativa* extracts have a range of MIC and isolates have a range of MIC of 0.2–2048 mg/mL, while the isolate has a range of MIC at 8–16 mg/mL.¹²³ The excellent MIC ranges are at 1–8 mg/mL.¹¹² The data showed that isolates from *N. sativa* had better activity than extracts against bacteria that cause biofilms. This is a reference that *N. sativa* isolates have great potential as an alternative to natural anti-biofilms (presented in Table 2).

Table 1 Bioactivity of *N. sativa* from Different Countries

No.	Regional Origin	Extract/Compound	Activity
1	Turkiye	Thymoquinone	Antioxidant ¹⁰⁴
2	Tunisia	Essential oil	Antibacterial; Anticancer; Anti-inflammatory and Antioxidant ¹⁰⁹
3	Tunisia	2-methyltetrahydrofuran (MeTHF) extract)	Antioksidan and Anti-inflamasi ¹⁰⁷
4	Pakistan	Methanol: Water Extract (30:40 v/v)	Antioxidant ¹⁰⁸
5	Iran	Oil BCS	Antioxidant ¹⁰⁵
6	Irak	Beta-d-glucopyranoside, alpha-d-mannopyranoside, methyl, diethyl phthalate, 12-octadecadienoic acid (Z,Z)-, and L-(+)-ascorbic acid 2,6-dihexadecanoate i	Antibacterial ¹¹⁰
7	Turkiye and Egypt	Supercritical fractionation extraction with carbon dioxide	Antibacterial, Antifungal, Antituberculosis ¹¹⁰
8	Local market confirmed Moroccan flora documents	Water extracts	Antioxidant ¹¹¹
9	Lokal market Australia	p-hydroxybenzoic acid extracts	Antioxidant ¹¹²
10	Indonesia	Oil	Breast anticancer ¹¹³
11	Canada	Thymoquinone	Breast anticancer ¹¹⁴
12	Local market in Zagazig	BCSO dan CSO	Antibacterial and Antioxidant ^{115,116}
13	China	Oil by SEF method	Antibacterial and Antioxidant ^{117,118}
14	Traditional Market Indonesia	Encapsulated ethanol extract	Antioxidant ¹¹⁹
15	Beni Mellal	BCSO	Antioxidant ¹²⁰

Abbreviations: BCS, black cumin seed; BCSO, black cumin seed oil; CSO, cumin seed oil; SEF, supercritical fluid extraction.

Table 2 Anti-Biofilm of *N. Sativa* Seed

No.	Regional Origin	Extract/Compound	Bacterial	Activity
1	Tunisia	Hexane extract ¹²⁴	<i>Staphylococcus aureus</i>	MIC: 38 µg/mL
2	Local Markets Indonesia	Ethanol extract ¹²⁵	<i>Streptococcus mutans</i>	MIC: 380 mg/mL
3		Thymoquinone ¹²⁶	<i>Staphylococcus aureus</i> ATCC 29213	MIC: 50 mg/mL MBEC: 50 mg/mL
4	Iran	Oil of <i>Nigella sativa</i> Linn. ^{127,128}	<i>Streptococcus mutans</i>	MIC: 2048 mg/mL MBC: 2048 mg/mL
			<i>Enterococcus faecalis</i>	MIC: 2048 mg/mL MBEC: 2048 mg/mL
5	Saudi Arabia and Syria	Oil of <i>Nigella sativa</i> Linn. ^{129,130}	<i>Staphylococcus aureus</i> ATCC 29213	Zona hambatan 24 dan 15 mm
6	Saudi Arabia	Water extract ¹³¹	<i>Staphylococcus aureus</i>	MIC: 6.5 mg/mL
			<i>Enterococcus faecalis</i>	MIC: 6.5 mg/mL
7	India	Methanol extract ¹³²	<i>Streptococcus mutans</i> (MTCC 497)	MIC: 0.2 mg/mL
		Water extract ¹³²	<i>Streptococcus mutans</i> (MTCC 497)	MIC: 0.3 mg/mL
8	Sigma-Aldrich	Thymoquinone ¹³³	<i>Streptococcus mutans</i>	MIC: 8–16 mg/mL

Conclusion

N. sativa seeds contain secondary metabolites such as essential oils, alkaloids, terpenoids, polyphenols and steroids. Extracts and isolates of *N. sativa* seeds from various countries have anticancer, antioxidant, antibacterial and anti-inflammatory activities that have been tested in vitro. On biofilm-causing bacteria, *N. sativa* has excellent activity. Through this article, it can provide information that *N. sativa* can be used as a potential drug candidate as an anti-biofilm and antibacterial oral pathogen that causes dental caries.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors declare no conflicts of interest.

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