

Effect of *Nigella Sativa L.* extracts against *Streptococcus mutans* and *Streptococcus mitis* in Vitro

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ABSTRACT

Background: Seeds of *Nigella sativa L.*, commonly known as black seed, have been used in traditional medicine. *Streptococcus mutans* and *Streptococcus mitis* are normal flora bacteria found in human oral cavity, which cause dental caries and bad breathe oder. This project considered as an explorer study for the inhibitory effect of *Nigella Sativa L.* seeds extract against *S. mutans* and *S. mitis*.

Material and methods: Two different extracts of *Nigella sativa L.* has been evaluated *in vitro* against *Streptococcus mutans* & *Streptococcus mitis*, the antibacterial activity was determined by the agar well diffusion method.

Results: The results showed the zone of inhibition was found 12.7mm and 10.4mm at a ethanol extract against *Strept. mutans* & *Strept. mitis* respectively, while the inhibition zone of ether extract was found 6.3mm and 5.1mm against *Strept. mutans* & *Strept. mitis* respectively.

Conclusion: Methanol extract was more effective in comparison with the ether extracts. The highest inhibition zone was observed with ethanol fraction and it inhibited the growth of two careiogenic bacteria.

Key words: *Nigella sativa L.*, well diffusion, Antibacterial activity. (J Bagh Coll Dentistry 2012;24(3):154-157).

INTRODUCTION

The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and diverse types of plants grow in different parts of the country ⁽¹⁾. Herbal medicine is still the mainstay of 75-80% of the whole population and the major part of traditional therapy involves the use of plant extract and their active constituents. Following the advent of modern medicine, herbal medicine suffered a setback, but during last two or three decades, advances in phytochemistry and in identification of plant compounds, effective against certain diseases have renewed the interest in herbal medicines ⁽²⁾. In recent years, human pathogenic microorganisms have developed resistance in response to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. Therefore, alternative antimicrobial strategies are urgently needed, and thus this situation has led to a re-evaluation of the therapeutic use of ancient remedies, such as plants and plant-based products ⁽³⁾. *Nigella sativa L.* is commonly known as which belongs to the botanical family of Ranunculaceae. *N. sativa* seeds have been used for nutritional and medicinal purposes in many Middle Eastern countries and other parts of the world ^(4, 5). *N. sativa* is considered a natural food additive and a condiment. Also, it had been used for medicinal purposes as a natural remedy in many ancient cultures, as those of Egypt's Greece and Rome ⁽⁶⁾.

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The *N. sativa* is the black seed medicine for every disease except death ^(7, 8).

Recently, many biological activities of *Nigella sativa L.* seeds have been reported, including: antioxidant, anti-inflammatory, anticancer and antimicrobial. *Nigella sativa L.* seeds contain a large amount of fixed oils ⁽⁹⁾ and the main constituent of the seed extract is thymoquinone ⁽¹⁰⁾. Several pharmacological effects have been attributed to active principles of *Nigella sativa L.* which includes thymoquinone, thymohydroquinone, dithymoquinone, thymol, carvacrol, nigellicine, nigellimine-x-oxide, nigellidine and alpha-hedrin ⁽¹¹⁾. The seeds or its oil is believed to have carminative, diuretic, lactagoge and vermifuge ⁽¹²⁾. *N. sativa* seeds have a wide spectrum of medicinal properties including antimicrobial, antihelminthic, antiinflammatory, analgesic, hypoglycemic, smooth muscles relaxant and immunostimulant activities ^(12,13).

The anti-microbial effects of *N. sativa* seeds against different pathogenic microbes were investigated. The volatile oil of *N. sativa* seeds, prepared by steam distillation, was proved to be more effective against many strains of bacteria, including those known to be highly resistant to drugs ⁽¹⁴⁾. The diethyl ether extract was found to cause concentration dependent inhibition of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and a pathogenic yeast *Candida albicans* ⁽¹⁵⁾. The methanol and chloroform extracts have high inhibitory effects against *S. aureus*, *P. aeruginosa* and *C. albicans* ⁽¹⁶⁾. The essential oil of the seeds have also dose-dependent antibacterial effects on Gram-positive

(*S. aureus*) and Gram-negative (*E. coli*) bacteria⁽¹⁷⁾.

Among the various oral-micro-organisms, *Streptococcus mutans* has been identified as a plaque-forming bacterium capable of producing dental caries in human⁽¹⁸⁾. Many attempts have been made to eliminate *S.mutans* from the oral flora.

The present study aimed to screen and evaluate antibacterial activity of two different extracts of *Nigella sativa* L. against *Streptococcus mutans* & *Streptococcus mitis*.

MATERIALS AND METHODS

Preparation of extracts:

Nigella sativa seeds were obtained from the local seed supplier; the seeds were crushed manually in a mortar with a pestle. A volume of 100 ml of distilled water was added to 20 g of dry powder. It was vortexed continuously until there was no further change in color of the solution. This solution was centrifuged for 15 min. The supernatant (brownish-orange in color) was filtered through Whatman filter No.1 using Buchner funnel and stored at 4 °C in sterile tubes until use.

Methanolic extraction:

10 grams of powdered plant was soaked separately in 50 ml of methanol and rotated on a rotary shaker at 150 rpm for 24 h. The extracts were filtered through Whatman filter paper no. 1 using Buchner funnel and the filtrates concentrated in vacuo at 40°C using a rotary evaporator and then stored in refrigerator at 4°C until used⁽¹⁷⁾.

Ether extract:

10 grams of seeds were placed to a Soxhlet and extracted with diethylether at 35 °C. Ether was evaporated after extraction by a rotary evaporator connected to a vacuum pump⁽¹⁷⁾.

Antibacterial activity:

The tested bacteria firstly isolated from saliva patients on mitis salivarius + bacitracin medium

and identification by API-20 Strep. System (bioMérieux, France). Secondly the antibacterial activity was determined using micro well dilution methods⁽¹⁹⁾, and by using the Kirby-Bauer method⁽²⁰⁾ bacterial cultures were first grown in nutrient broth at 37°C for 18-24 h incubated till turbidity became equivalent to McFarland 0.5 turbidity standard was obtained. The inocula of the respective bacteria were streaked on to the Muller Hinton agar (Oxoid) plates using a sterile swab in order to ensure a uniform thick lawn of growth following incubation. Wells of 6 mm in diameter were formed on to nutrient agar plates using a sterile cork borer. The dried plant extracts were dissolved in dimethylsulfoxide (DMSO) to give a concentration of 30 mg/ml. Wells were cut into the agar and filled with 75 µl of the plant extracts and one well with (DMSO) as negative control. Inoculated plates were incubated at 37 °C for 18- 24hrs. Finally, the plates were incubated at 37°C for 18-24 h and the resulting diameters of zones of inhibition were measured. These studies were performed in triplicate for each plant extract.

Statistical analysis: The results were calculated as mean diameter of inhibition zone in mm ± standard deviation (mean ± SD).

RESULT AND DISCUSSION

Spices are frequently used as an active ingredient in certain medicines and reported to possess a number of pharmacological effects to treat different human ailments⁽²¹⁾.

Two different extracts of *Nigella sativa* L. has been evaluated *in vitro* against two oral bacteria, which are known as causative agents of dental caries in humans. In the plates of *Strep. mutans* and *Strep. mitis* no inhibition zones seen around the methanol wells which were used as negative control. Among the different extracts, the methanolic extract showed most pronounced antibacterial activity. The inhibition zone around the discs containing ether and ethanol extract of *N.sativa* seeds shown in Table (1).

Table 1: The diameter zone of inhibition (mm) around discs containing the two *N.sativa* seeds extracts against *Streptococcus mutans* & *Streptococcus mitis*

Extract	Zone of inhibition (mm)	
	<i>Strep. mutans</i> (mm) ±SD	<i>Strep. mitis</i> (mm) ± SD
Ethanol	(12.7) ± 2.1	(10.4) ± 0.9
Ether	(6.3) ± 0.6	(5.1) ± 0.59
Negative control	-	-

SD = Standard deviation.

Methanol extract was more effective in comparison with the ether extracts. The highest inhibition zone was observed with ethanol

fraction and it inhibited the growth of two cariogenic bacteria figure (1&2).

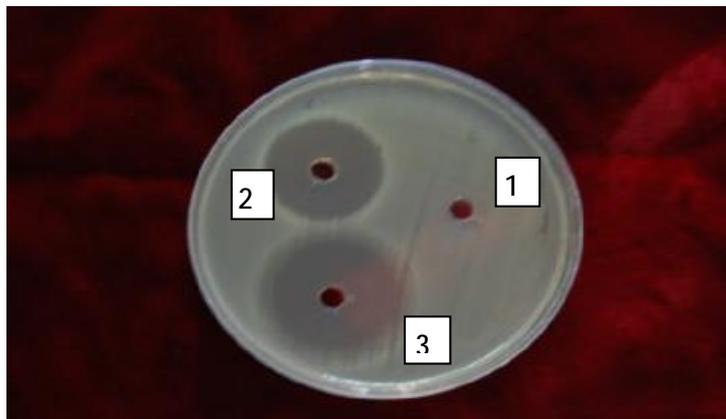


Figure 1: Inhibition zone of *Nigella sativa* L. & against *Strep. mutans*. 1=negative control. 2= ether extract. 3=Methanol extract.

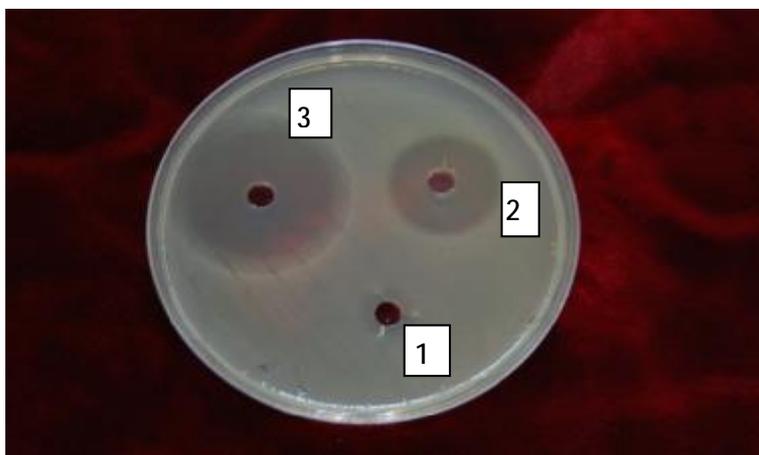


Figure 2: Inhibition zone of *Nigella sativa* L. & against *Strep. mitis*. 1=negative control. 2= ether extract. 3= Methanol extract.

It is interesting to note that G+ve bacterial isolates were sensitive to ether and ethanol extract of *N. sativa* seeds. Present study is in agreement with ⁽¹⁴⁾ who reported that *N. sativa* extracts produce antibacterial activity against a broad range of microbes and especially G+ve strains and multiple antibiotic resistant bacteria. This result also correlates with ⁽²²⁾ who reported that Thymoquinone exhibited potent growth inhibitory effect against Gram-positive bacteria,

During extraction process, solvents diffuse into the solid plant material and soluble compounds of similar polarity ⁽²³⁾. The polarity of solvent affects quantity and composition of secondary metabolite of an extract. Traditional healers primarily use water for extract preparation from plant extracts but organic solvents have been found to give more consistent antimicrobial activity compared to water extracts ⁽²⁴⁾. Most other bioactive

compounds including phenols are generally soluble in polar solvents such as methanol. Many bioactive components are not water soluble and thus organic solvent extracts of plants have been found to be more effective ⁽²⁵⁾. Methanol, ethanol and water are the most commonly used solvents for antimicrobial investigations. ⁽²⁶⁾ Since most of the identified components from plants active against microorganisms are aromatic or saturated organic compounds, they are most often obtained through ethanol or methanol extraction. The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols. One of these active ingredients is Thymoquinone (volatile oil of these seeds) and Melanine (fixed oil) ⁽²⁷⁾. Some investigations performed to show the possibility of antimicrobial and antibacterial activities of

these seeds using their extracts or oil⁽²⁸⁾. Generally, higher levels protein and carbohydrate content of the extract had better antimicrobial activities⁽²⁹⁾. Many proteins are involved in the microbial defense mechanism of plants. Puroindoline is the main component of a new family of proteins that has been suggested to exert an antimicrobial activity in plant seeds^(30,31).

The study provides support to the antibacterial potential of *N. sativa* extracts as a potent antibacterial agent.

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