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# ANTIBACTERIAL ACTIVITY OF *NIGELLA SATIVA* SEED OIL AGAINST COMMON PATHOGENS IN DENTAL PLAQUE

**MITHRA S[1*], RAMANI P[2], SHERLIN HJ[3], GHEENA S[4], RAMASUBRAMANIAM A[5], JAYARAJ G[6], DON KR[7] AND SANTHANAM A[8]**

**1:** MDS, Post Graduate student, Department of Oral and Maxillofacial Pathology, Saveetha Dental College & Hospitals, SIMATS, Chennai

**2:** Professor and Head, Department of Oral and Maxillofacial Pathology. Saveetha Dental College & Hospitals, SIMATS, Chennai

**3:** Professor, Department of Oral and Maxillofacial Pathology, Saveetha Dental College & Hospitals, SIMATS, Chennai

**4:** Reader, Department of Oral and Maxillofacial Pathology, Saveetha Dental College & Hospitals, SIMATS, Chennai

**5:** Reader, Department of Oral and Maxillofacial Pathology, Saveetha Dental College & Hospitals, SIMATS, Chennai

**6:** Reader, Department of Oral and Maxillofacial Pathology, Saveetha Dental College & Hospitals, SIMATS, Chennai

**7:** Senior Lecturer, Department of Oral and Maxillofacial Pathology, Saveetha Dental College & Hospitals, SIMATS, Chennai

**8:** Senior Lecturer, Department of Oral and Maxillofacial Pathology, Saveetha Dental College & Hospitals, SIMATS, Chennai

***Corresponding Author: Dr. Mithra S: E Mail: celestinemithra14@gmail.com**

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## ABSTRACT

Background: Dental Plaque is a sticky coat that contains tons of bacteria which further leads to periodontal problems. And periodontitis is one of the most common oral disease among the Asian population. Anti-inflammatory, immune modulation, anticancer,

antioxidant, anti-glycemic and hepatoprotective activities are some biological activities of *Nigella sativa* seed crude extract.

Aim: The aim of this study was to formulate oil-in-water emulsion of *Nigella sativa* seed oil extract and to investigate its antimicrobial activity against common the pathogens present in dental plaque so as to prevent periodontal problems.

**Materials and methods:** Samples of dental plaque were collected from 8 patients and Streptococcus and Staphylococcus cultures were grown in Blood Agar & Mannitol Salt Agar. Various biochemical tests were done to determine the bacterial species present in the given sample. Then Antibacterial Activity of *Nigella sativa* seed oil in water emulsion was done using Micro-Broth Dilution method using Resazurin dye.

**Result**: Antibacterial activity of *Nigella sativa* as oil in water emulsion was present for *staphylococcus* species in a Minimum Inhibitory Concentration of 1:5 and 1:6, but for streptococcus the activity was less when compared to *staphylococcus*.

**Conclusion**: Based on the result of this study, it can be concluded that *Nigella sativa* seed oil possesses antibacterial activity for staphylococcus species when compared to streptococcus present in dental plaque.

**Keywords: *Nigella sativa*, periodontitis, plaque, blood agar, mannitol salt agar, resazurin**

## INTRODUCTION

Periodontitis is a chronic inflammatory disease of the gums resulting from an opportunistic infection of endogenous plaque biofilm. It usually manifests as a worsening of gingivitis and then, if untreated, with loosening and loss of teeth **[1]**. Periodontal infection is initiated by specific invasive oral pathogens that colonize dental plaque biofilms on tooth surface and host immune response to inflammation plays a central role in disease pathogenesis. Periodontal diseases are recognized as infectious processes that require bacterial presence and a host response and modified by other local, environmental and genetic factors. Dental Plaque is a biofilm that adheres to the tooth surface, this contains many number of pathogens and the most common pathogens present in plaque are the streptococcus and staphylococcus species **[2]**.

*Nigella sativa* is an annual flowering plant. It grows to 20–30 cm tall and with linear lanceolate leaves. The delicate flowers have 5-10 petals. The fruit of this plant is a large inflated capsule composed of 3-7 united follicles, that each of them has numerous seeds. The black

coloured seeds are flattened, oblong and angular, funnel shaped, with the length of 0.2 cm and 0.1 cm wide. This plant is known by numerous names, for example black cumin (English), black caraway seeds (USA), shonaiz (Persian) and kalajira (Bangali) **[3]**. Exhibiting good antimicrobial activity, *Nigella sativa* seed, known as black cumin, has been widely used as herbal medicines all over the world for the treatment and prevention of a number of diseases.

Antimicrobial activity of *N. sativa* seed against various strains of Streptococcus has also been reported previously. Anti-inflammatory, anticancer, antioxidant, immunomodulation, anti-glycemic and hepatoprotective activities are some biological activities of N. sativa seed crude extract or essential oil that have been scientifically revealed, with thymoquinone as the main bioactive constituent **[4-6]**. The black seeds contain 36–38% fixed oil, with proteins, alkaloids, saponins and essential oils making up the rest of the composition. Although black seed oil has been reported to possess many antimicrobial activities, antioxidant activities, antitumor activities and a stimulatory effect on the immune system, its full potential as an antimicrobial agent has not been exploited **[7]**.

Microorganisms now a days are more resistant to existing antibiotics that led to an increase in the use of medicinal plants that shows beneficial effects for various infectious diseases. Recently, the antibiotics resistance has become a serious global health concern and also a huge economic burden in the community by increasing the treatment cost.

The development of new antibiotics, which are costly and time-consuming process, has become useless, as pathogens rapidly develop resistance to these new antibiotics. This has led to an increased interest facts in searching for effective alternatives for the current antibiotics. Medicinal plants as always appears to be the best alternative source for any new antimicrobial drugs. The current study was conducted to investigate the antibacterial activity of *Nigella sativa* oil in water emulsion against pathogenic isolates of bacteria in dental plaque **[8]**.

## MATERIALS AND METHODS

Plaque samples were collected from 8 patients using periodontal probes and then it was dispensed to Eppendorf Tube filled with 1ml of Distilled water, The collected samples were then cultured using Blood Agar and Mannitol Salt Agar and then Biochemical tests were carried out to find out the most prevalent pathogens present in the samples.

## BLOOD AGAR PLATE

Nutrient agar 2.8 gm and 1 gm of agar were dissolved in 100 ml of distilled water and then sterilized. 5 ml of fresh blood mix was added without air bubble formation. Then it was poured in sterile petri plate and was allowed to solidify for 30 minutes. 100 µl of sample was spread into the solidified agar medium using L-rod. The sample plates were then incubated at 37°C for 24 hours. The colonies were then studied for its characteristics.

## MANNITOL SALT AGAR

Mannitol salt agar 111.02 gm and 1 gm of agar was dissolved in 100 ml of distilled water and then sterilized. Then it was poured in sterile petri plate and was allowed to solidify for 30 minutes. 100 µl of sample was spread into the solidified agar medium using L-rod. The samples plates were then incubated at 37°C for 24 hours. The colonies were then studied for its characteristics.

## BIOCHEMICAL TESTS

Various Biochemical Tests were performed to identify the species of bacteria present in the given samples.

## INDOLE TEST

Tryptone broth was inoculated with the test isolates and incubated at 37°C for 24-48 hours. About 0.2-0.3 ml of Kovac's reagent was then added to the test tube, shaken and allowed to stand.

**Tryptone Broth**

| | |
|---|---|
| **Tryptone** | **10.0 gms** |
| **Distilled Water** | **1000 ml** |

**Kovac's Reagent**

| | |
|---|---|
| **Para-dimethyl amino Benzaldehyde** | **5 gms** |
| **Butyl alcohol** | **75 ml** |
| **Conc. hydrochloric acid** | **25 ml** |

## METHYL RED TEST

Methyl red – vogesproskauer (MR-VP) broth inoculated with the culture was incubated for 24-48 hrs at 37°C. pH is 7.2

**Glucose – Phosphate broth (MR – VP)**

| | |
|---|---|
| **Buffered Peptone** | **7.0 gms** |
| **Dextrose** | **5.0 gms** |
| **Dipotassium phosphate** | **5.0 gms** |
| **Sodium chloride** | **5.0 gms** |
| **Distilled Water** | **1000 ml** |
| **Methyl red** | **0.1 g** |
| **95 % ethanol** | **300 ml** |
| **Distilled water** | **200 ml** |

**Methyl Red reagent**

## VOGES - PROSKAUER TEST

MR - VP medium inoculated with culture was incubated at 37°C for 24-48 hrs. After incubation, 3 ml of Barrit's reagent A and one ml of Barrit's reagent B was added. The tubes were shaken and allowed to stand for 15 minutes and observed for colour change.

**Barrit's Reagent**

| | |
|---|---|
| **5% alpha naphthol** | **5.0 gms** |
| **Absolute ethanol** | **95 ml** |

**Reagent B**

| | |
|---|---|
| **Potassium hydroxide** | **40 gms** |
| **Creatine** | **3 gms** |
| **Distilled Water** | **1000 ml** |

## CITRATE UTILIZATION TEST

Citrate utilization test was used to detect the ability of an organism to utilize citrate as the sole carbon source for its growth, lightly inoculate Simmon Citrate Agar (Hi media, Mumbai) with a loop (pure culture) of culture. Incubate at 37°C for 24 hrs.

**Preparation of Citrate Agar**

| | |
|---|---|
| **Sodium Chloride** | **5 gms** |
| **Magnesium Sulphate** | **0.2 gms** |
| **Ammonium dihydrogen phosphate** | **1 gm** |
| **Dipotassium phosphate** | **1 gm** |
| **Sodium Citrate** | **2 gms** |
| **Bromothymol blue** | **0.08 gm** |
| **Agar** | **15 gms** |
| **Distilled Water** | **1000 ml** |
| **pH** | **6.8** |

## TRIPLE SUGAR IRON TEST

The Triple Sugar Iron (TSI) test is a test to find the ability of a microorganism to ferment sugars and to produce hydrogen sulfide. Acidic byproduct gives yellow color whereas alkaline by products gives red color. Black color indicated hydrogen sulphide formation. Gas production is indicated by lifting of butt from surface of test tube. TSI medium inoculated with the culture was incubated for 24 hrs at 37°C. The pH is 6.8

**Triple sugar iron agar medium**

| | |
|---|---|
| **Beef extract** | **3 gms** |
| **Peptone** | **20 gms** |
| **Yeast Extract** | **3 gms** |
| **Lactose** | **10 gms** |
| **Sucrose** | **10 gms** |
| **Dextrose monohydrate** | **1 gms** |
| **Ferrous sulphate** | **0.2 gms** |
| **Sodium chloride** | **5 gms** |
| **Sodium thiosulphate** | **0.3 gms** |
| **Phenol red** | **0.024 gms** |
| **Agar** | **12.0 gms** |
| **Distilled water** | **1000 ml** |

## UREASE TEST

When urea is utilized, ammonia is formed during incubation which makes the medium alkaline, showing a pink-red colour. Urease medium inoculated with the culture was incubated for 24 hrs at 37°C.

**Urease agar medium**

| | |
|---|---|
| **Dextrose** | **1 gms** |
| **Peptic digest of animal tissue** | **1.5 gms** |
| **Sodium chloride** | **5 gms** |
| **Monopotassium phosphate** | **2 gms** |
| **Phenol red** | **0.012 gms** |
| **Agar** | **15 gms** |
| **Final pH (at 25°C)** | **6.8±0.2** |

## CATALASE TEST

A small amount of culture was placed over a clean slide. A drop of 3% hydrogen peroxide was placed over the culture and observed for effervescence. The production of effervescence showed the ability to produce the enzyme catalase.

## COAGULASE TEST

A small amount of culture was placed over a clean slide. A drop of serum was placed over the culture and observed for coagulation. The coagulation showed the ability to produce the enzyme coagulase.

### Gram Staining

Bacterial smears of 16-18 hrs old cultures were made on clean grease free slides, heat fixed and stained as follows. The slide was flooded with crystal violet solution for a minute, drained and rinsed

with water; followed by Grams iodine solution for one minute, drained and rinsed with water. Then it was decolorized with ethyl alcohol for 30 Sec and later counterstained with safranin for one minute and observed under an oil immersion microscope.

**Crystal Violet**

| | |
|---|---|
| **Crystal violet** | **2.0 g in 20 ml 95% ethanol** |
| **Ammonium Oxalate** | **8.0 g in 80ml distilled water.** |

**Grams Iodine**

| | |
|---|---|
| **Iodine** | **1.0 g** |
| **Potassium Iodine** | **2.0 g** |
| **Distilled Water** | **300 ml** |

**Acetone Alcohol**

| | |
|---|---|
| **95% ethanol** | **70 ml** |
| **Distilled water** | **30 ml** |

**Gram's Safranin**

| | |
|---|---|
| **Safranin** | **0.25** |
| **95% ethanol** | **10 ml** |
| **Distilled Water** | **100 ml** |

**ANTIBACTERIAL EVALUATION**

**Determination of Minimum inhibitory concentration (MIC) using Resazurin (Micro titre Assay):**

**Preparation of resazurin solution**

The resazurin solution was prepared by dissolving 270 mg in 40 mL of sterile distilled water. A vortex mixer was used to ensure that it was a well-dissolved and homogenous solution.

**Procedure**

Test was carried out in a 96 well Plates under aseptic conditions. A sterile 96 well plate was labeled. A volume of 100 µL of sample was pipetted into the first well of the plate. To all other wells 50 µL of nutrient broth was added and serially diluted it. To each well 10 µL of resazurin indicator solution was added. 10 µL of bacterial suspension was added to each well. Each plate was wrapped loosely with cling film to ensure that bacteria does not become dehydrated. The plate was incubated at 37 °C for 18–24 h. The colour change was then assessed visually.

**RESULTS:**

**BIOCHEMICAL TESTS: (Table 1)**

**INDOLE TEST:**

The formation of brown ring on the surface of the broth confirmed the production of indole.

**METHYL RED TEST:**

The appearance of a red colour on addition of methyl red solution was considered as positive.

**VOGES - PROSKAUER TEST:**

The development of red colour was considered as positive.

**CITRATE UTILIZATION TEST:**

Blue colour growth is recorded as positive and absence of growth as negative.

**TRIPLE SUGAR IRON TEST:**

Acidic byproduct gives yellow color whereas alkaline by products gives red color both acidic and alkali indication is marked positive. Black color indicated hydrogen sulphide formation is marked negative.

**UREASE TEST:**

Indication of Pink – Red colour is marked positive.

**CATALASE TEST:**

The production of effervescence showed the ability to produce the enzyme catalase.

**OXIDASE TEST:**

The organism spotted on oxidase disc was observed for colour change (blue or purple)

**COAGULASE TEST:**

The coagulation showed the ability to produce the enzyme coagulase.

**Gram Staining:**

Gram staining was positive for all three samples.

**ANTIBACTERIAL ACTIVITY TEST:**

**(Table 2)**

If activity is present in a sample then the colour changes to purple. If no activity is present it stays colorless or changes to pink. The lowest concentration at which purple colour remains constant was taken as the MIC value. It was found that the culture grown in mannitol salt agar (white and yellow sample) had activity in which it had purple colour, but the one grown in blood agar did not have any activity. The control standard kept was the Streptomycin group.

**Table 1: Biochemical Tests**

| TEST | WHITE | YELLOW | BLOOD |
|---|---|---|---|
| Indole | - | - | + |
| MR | - | - | - |
| VP | - | - | + |
| TSI | - | - | + |
| Urease | + | + | + |
| Citrate | - | + | + |
| Catalase | + | + | + |
| Oxidase | + | - | - |
| Coagulase | - | + | - |
| Gram staining | Positive cocci | Positive cocci | Positive cocci |

**Table 2: Antibacterial Activity Test**

| S. No. | Microorganisms/ sample | Growth of inhibition | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Volume 50 µl | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Positive | Culture |
| | | Direct | 1:1 | 1:2 | 1:3 | 1:4 | 1:5 | 1:6 | 1:7 | control 10µg | |
| | Sample | | | | | | | | | | |
| 1 | White | - | - | - | - | - | - | - | + | - | + |
| 2 | Yellow | - | - | - | - | - | - | + | + | - | + |
| 3 | Blood agar culture | - | + | + | + | + | + | + | + | - | + |

**Table 2: MIC**

| Microorganisms/sample | MIC Value |
|---|---|
| | |
| White | 1:6 |
| Yellow | 1:5 |
| Blood agar culture | Direct sample (1) |

**Figure 1: Culture Grown in Blood agar**

**Figure 2: Culture Grown Mannitol Salt agar**

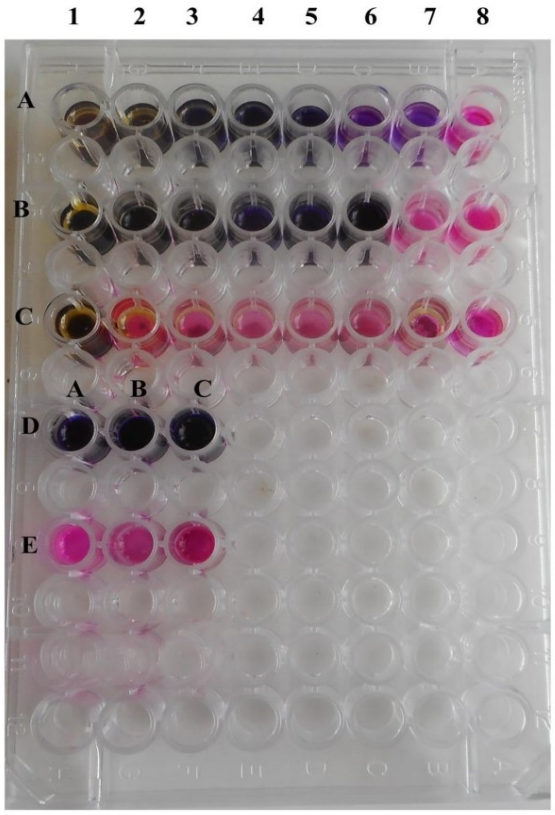


**Figure 3: Antibacterial using Micro-broth Dilution method with Resazurin**

**DISCUSSION**

The strong antibacterial activity shown in this study indicated that emulsification process did not significantly affect antibacterial activity of *N. sativa* seed oil extract. As one of widely used lipophilic compound delivery systems, oil emulsification is able to enhance their solubility in aqueous-based system, increases the compound stability and somehow improves the biological activity. Facing the multi-drug resistant pathogenic bacteria, discovery and development of new antimicrobial agents have already become a global concern.

In other studies, it has been proved that the antibacterial activity is present in streptococcus species[4] but In this study with the use of resazurin dye method there was less activity towards streptococcus species but at the same time the activity was very much significant for staphylococcus species.

This study highlighted the promising antimicrobial activity of *N. sativa* seed oil extract against bacteria present in dental plaque. Thus, it could further be used for oral remedy to prevent and/or cure the common oral diseases. The antibacterial effect synergism of *N. sativa* seed essential oil with antibiotic was reported in several previous studies, therefore it may be used in combination with antibiotic. Further investigation is needed in order to investigate the cytotoxic effect of *N. sativa* seed regarding to human use.

**CONCLUSION**

Based on the result of this study, it can be concluded that *Nigella sativa* seed oil possesses antibacterial activity and it can be used in therapeutics after further investigations on its cytotoxic effects.

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