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Comparison of the Antimicrobial Activity of *Nigella sativa* Aqueous and Oil Extracts

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ABSTRACT

Aims *Nigella sativa* is recognized as a black seed. It is a grassy plant relating to the Ranunculaceae family. There are various reports regarding this plant's pharmacological and biological action, like antihypertensive effects, antibacterial, anticancer, antioxidants, antifungal, pain alleviating, anti-inflammatory, antidiabetic, and immune-modulatory effects. This study aimed to compare the anti-microbial activity of aqueous and oil extract of *Nigella sativa* against selected gram-positive and gram-negative bacteria.

Materials & Methods *Nigella sativa* aqueous and oil extracts were gathered via a retail food shop (Al-Hilla) 2018. Gram-positive (*Staphylococcus aureus; Streptococcus pneumonia; Streptococcus pyogenes*) and Gram-negative (*Salmonella typhi; Escherichia coli; Pseudomonas aeroginosa*) isolates (obtained via clinical specimens) were utilized.

Findings *Nigella sativa* aqueous and oil extracts showed a maximum inhibition zone against *E. coli* and minimum inhibition against *S. pyogenes*.

Conclusion *Nigella sativa* acts against gram-positive as well as gram-negative bacterial isolates.

Keywords Antimicrobial Activity; *Nigella sativa*; Medicinal Plant; Minimum Inhibitory Concentration

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Introduction

One of the successful areas in human therapeutics for treating bacterial infections in the 20th century was the development of antibiotics and antibacterial agents. A huge range of antibacterial agents have been discovered, and different antibiotics can be used to treat major infectious diseases [1]. However, the usage of antibacterial chemotherapeutics is becoming more restricted for some reasons, including (a) capability of increased resistance to antibiotics in bacteria immediately after their introduction; and (b) side effects in most antibodies. Therefore, it is essential to replace newer drugs with a lower rate of resistance and toxicity [2].

Since ancient times, medicinal plants have been used to treat human diseases. Today, herbal medicine is the mainstay of 75-80% of the whole population, and a considerable part of traditional therapy includes the use of plant extract and its constituents. Following the advent of modern medicine, herbal medicine declined. Still, over the last two or three decades, herbal medicines have been taken into account due to the advances in phytochemistry and the identification of effective plant compounds against certain diseases [3].

The medicinal plant can change pathological and physiological processes and treat or prevent diseases. In recent years, a significant increase was observed in the use of medicinal plants, compared with the chemical drugs, considering several factors, such as low cost, no need to refer to healthcare professionals, easy access without prescription, and fewer side effects for the treatments with natural products. Based on the report of the world health organization, about 80% of people benefit from herbal remedies [4].

Famous explanations for the effectiveness and use of medicinal plants significantly contribute to the acceptance of their therapeutic possessions, even though their chemical components are entirely not identified [5]. There are greater than 20,000 species of all recognized medicinal plants internationally utilized listed according to WHO (World Health Organization) [6]. Few compounds segregated via these plants demonstrated to be very impactive inhibitory medicine and utilized to cure composite cases like a cancer disease [7].

Nigella sativa is recognized as black seed. It is a grassy plant relating to the Ranunculaceae family, which have born utilized in North Africa, Southwest Asia, and Southern Europe, and it is cultivated in several countries universally, like the Mediterranean zone, Syria, Saudi Arabia, Turkey, South Europe, India, Pakistan, Middle East [8]. The height of the *Nigella sativa* herb is 20–25 cm. The leaves are divided into linear segments 2-3 cm long. The leaves are in pairs on opposite sides of the stem. The upper leaves are longer than the lower leaves and have petioles, and the flowers grow terminally on their branches. The flowers are mostly yellow, white, light blue, pink, or lavender and have 5 to 10 petals [9]. Its ripe fruit contains tiny seeds, dark black, known as "Habba Al-Sauda" or "Habba Al-Barakah" in Arabic and black seed in English [10]. The *N. sativa* is usually planted between November and April, and germination continues about 10-15 days after sowing the seeds. The flowering and fruiting time of the plant is generally from January to April [11].

The necessary crucial *N. sativa* seeds phytoconstituents involve α-pinene, dithymoquinone, thymohydroquinone, sesquiterpene longifolene (1-8%) thymol, t-anethol (1-4%), carvacrol (6-12%), 4 terpineols (2%-7%), p-cymene (7-15%), thymoquinone (30-18%), etc. additionally to low quantities of few other components like citronellol, carvone, and limonene. Also, alkaloids are present in seeds involving nigellicimine types such as nigellicimine-N-oxide and isoquinoline alkaloids and types of pyrazole alkaloids such as nigellidine and nigellicine. In addition, they have water dissoluble saponins, alpha-hederin, and triterpene as crucial anticancer compounds [12-14].

Many bioactive components are not water-soluble, and organic solvent extracts of plants are more effective [15]. Methanol, ethanol, and water are the commonly used solvents for antimicrobial considerations. Most of the identified components of plants active against microorganisms are aromatic or saturated organic compounds obtained through ethanol or methanol extraction. The curative properties of medicinal plants are due to various secondary metabolites such as alkaloids, flavonoids, lycosides, phenols, saponins, sterols. One of these active ingredients is Melanine (fixed oil) and Thymoquinone (volatile oil of these seeds) [16]. Some investigations were performed to show the possibility of antimicrobial and antibacterial activities of these seeds using their extracts or oil [17]. Higher protein and carbohydrate content levels of the extract had better antimicrobial activities [18]. Many proteins are involved in the microbial defense mechanism of plants. Puroindoline is the main component of proteins suggested to exert antimicrobial activity in plant seeds [3].

Ahmad *et al*. [19] have shown that methanolic extract of *N. sativa* has the strongest antifungal effect against various pathogenic yeasts, followed by chloroform and ethanolic extracts.

The phytochemical studies have revealed that the plant seeds contain two classes of alkaloids, including isoquinoline alkaloids such as nigellimine-N-oxide and pyrazole alkaloids such as nigellidine and nigellicine. *N. sativa* seeds are rich in unsaturated fatty acids such as linoleic acid, oleic acid, and palmitic acid. Other components of the seeds include saponins, indazole-type alkaloids, flavonoids, vitamins, cardiac glycosides, and some important minerals like calcium, phosphorus, and iron [20].

There are various reports regarding this plant's pharmacological and biological action, like antihypertensive effects, antibacterial, anticancer, antioxidants, antifungal, pain alleviating, anti-inflammatory, antidiabetic and immune-modulatory effects [21, 22].

Thymoquinone (TQ) is crude extract's active substance of NSO (Nigella sativa oil) that has anti-inflammatory/antioxidant efficiency; it is recognized as a crucial anti-mutagenic, anti-oxidant and anti-cancer agent [23].

It was revealed that the diethyl ether extract causes concentration-dependent inhibition of staphylococcus aureus, pseudomonas aeruginosa, escherichia coli, and a pathogenic yeast candida Albicans. The chloroform and methanol extracts have high inhibitory effects against *P. aeruginosa*, *C. albicans*, and *S. aureus* [24].

Chronic toxicity studies in laboratory animals have reported that *N. sativa* seed, its oil, and thymoquinone, the most abundant and widely studied active ingredient, are safe, especially when used orally [25, 26].

N. sativa has been shown to be very effective in treating patients infected with viruses such as human immunodeficiency virus (HIV) and hepatitis C virus (HCV) in various clinical trials [27].

Based on the previous studies, *N. sativa* seeds have anti-microbial effects against different pathogenic microbes. The seeds' essential oil also has anti-bacterial effects on gram-negative and gram-positive bacteria. The more anti-bacterial effect of fixed oil has been observed against gram-positive than gram-negative bacteria. Topozada *et al*. [28] first reported the antibacterial effect of the phenolic fraction of *N. sativa* oil. El-Fatatry [29] isolated thymohydroquinone from the volatile oil of *N. sativa*, which was found to have high activity against gram-positive microorganisms, including *Staphylococcus aureus*.

The developing microbial resistance to antimicrobial agents is a serious problem [30]. The in vivo anti-microbial effect of the aqueous extract of *N. sativa* seeds was investigated in animals. Most studies on the anti-microbial potential of plant extracts, active ingredients and/or chemical drugs have been tested on experimental animals [31]. The anti-microbiological potential of methanol extract, n-Hexane extract, and water decoction of *Nigella sativa* seed was seen in different geographies. They observed the major differences in antimicrobial activity amongst the same solvent extract from a different origin. Extracts of *Nigella sativa* seeds from India were found to be far superior to other Nigella sativa seeds from other geographies. Differences in efficacy could be due to exposure to environmental conditions in particular geographies. The effects of exposure to heat, light and age of seed can contribute to variation in active constituents, which would affect its antimicrobial activity.

Experimental studies have revealed that *N. sativa* extracts have synergistic effects on eradicating *E. coli* besides antibiotics such as gentamicin, streptomycin, chloramphenicol, doxycycline, ampicillin, cephalexin, and nalidixic acid terbinafine [18]. Also, *N. sativa* has inhibitory effects on the growth of bacteria such as *Yersinia enterocolitica*, *Listeria monocytogenes*, *Corynebacterium pseudotuberculosis*, *Corynebacterium renale*, *Brucella abortus*, *Pasteurella multocida*, *Mannheimia haemolytica*, *E. coli*, *Trueperella* (Arcanobacterium) *pyogenes*, and *S. aureus* [32]. Another study has shown that NSO has an anti-staphylococcal effect comparable with antibiotics such as ceftazidime, cefuroxime, cefaclor, and cefamandole [33]. Moreover, N. sativa methanolic extract has the strongest anti-fungal effect. It exhibits an inhibitory effect against candidiasis in mice. In a study, antidermatophyte activity of *N. sativa* ether extract and thymoquinone (TQ) was examined against eight species of dermatophytes: four species of *Trichophyton rubrum* and one each of Trichophyton interdigital, *Trichophyton mentagrophytes*, *Epidermophyton floccosum*, and *Microsporum canis* [34]. Another study investigated the anti-oxidant and anti-schistosomal activities of garlic extract (GE) and NSO. The results have demonstrated that protection with GE and NSO significantly ameliorate the anti-oxidant capacity of schistosomiasis mice compared to the infected-untreated ones and prevent the hematological and biochemical changes [10]. In addition, Mahmoud *et al*. have studied the effects of NSO in liver damage induced by S. mansoni infection in mice. Infection with S. mansoni produces a pronounced elevation in the mouse serum activity of ALT, GGT, with a slight increase in AP level, while reducing serum albumin level and administration of NSO succeeds to correct the changes in ALT, GGT, AP activity, as well as the Alb content in serum [35].

In the present study, aqueous extract and oil of *Nigella sativa* were evaluated for their anti-microbial activity against selected gram-positive and gram-negative bacteria.

Material and Methods

Nigella sativa aqueous extracts and oils were gathered via a retail food shop (Al-Hilla) 2018. The extracts of *Nigella sativa* were prepared in 100ml DW (distilled water) and soaked 30gm powder of *Nigella sativa* in it, and permitted to sit for 72hr by filtration; it was sterilized (utilizing 0.45 millipore filter paper) [36].

Isolates of Bacteria

Gram-positive (*Staphylococcus aureus*; *Streptococcus pneumonia*; *Streptococcus pyogenes*) and Gram-negative (*Salmonella typhi; Escherichia coli; Pseudomonas aeroginosa*) isolates (obtained via clinical specimens) were utilized. These microorganisms were excited and cloned three consecutive times on nutrient agar (NA), and

nutrient agar slants were kept at 4°C. The recognition of these microorganisms was established by utilizing traditional biochemical analysis [37].

***in vitro* testing of antimicrobial activity utilizing assay of agar well diffusion**

A loop full of isolated bacterial growth was inoculated into NB (nutrient broth) and incubated for 18 hours at 37°C. Dilution of bacterial suspension was done with normal saline. Turbidity was compared with the standard tube (0.5 McFarland) to submit a consistent suspension including 1.5×108CFU/ml. Immerse swab of cotton into adjustment suspension and streaked the complete agar surface of Mueller-Hinton agar. These plates were kept at room temperature for 5-15 minutes to dry. Four wells were created on an agar medium of 5mm diameter using a cork borer, and 0.1ml extracts were added in wells. The zone inhibition size was calculated from well to plate's edge for growth inhibition.

Assay of antibacterial activity

The antibacterial action was detected by diffusion of agar disc [37]. Plates of agar were inoculated with the tested organism's 0.1 ml broth culture and were spread with a sterile glass spreader. The discs of antibiotic ciprofloxacin were added to the agar plate's center (in triplicates, the plates were performed). All tested organisms plates were then incubated overnight at 37°C. After incubation of 24hr, every extract was estimated for inhibition zone for all isolates. The inhibition zone diameter was calculated using an mm (millimeter) scale.

Findings

The antimicrobial activity of the aqueous extract of *Nigella sativa* showed a maximum inhibition zone against *E. coli* and minimum inhibition against *S. pyogenes* (Figure 1).

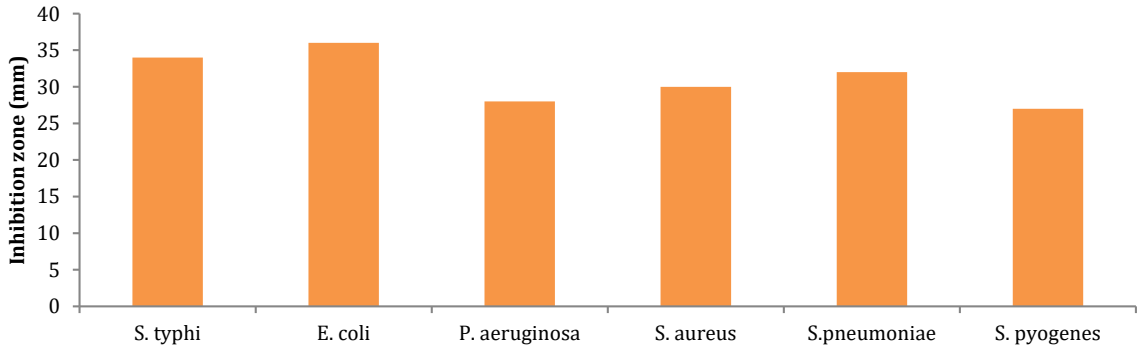

Figure 1) Antimicrobial activity of powder of *Nigella sativa* by agar well method

The antimicrobial activity of *Nigella sativa* oil was similar to the aqueous extract. It showed a maximum inhibition zone against *E. coli* and minimum inhibition against *S. pyogenes* (Figure 2).

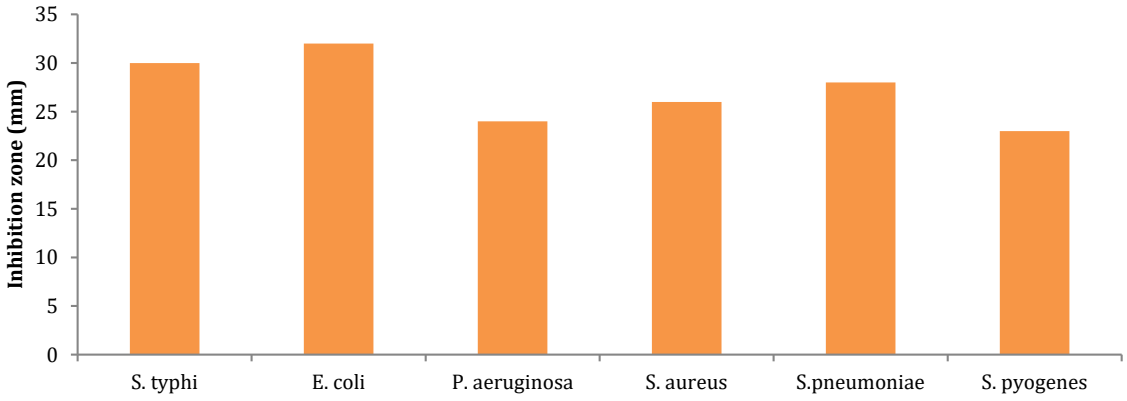

Figure 2) Antimicrobial activity of *Nigella sativa* oil by agar well method

The estimated MIC of *Nigella sativa* aqueous and oil extracts on the bacterial isolates was similar (p>0.05; Table 1).

Table 1) Effect of *Nigella sativa* extract and oil on the bacterial isolates estimated by MIC

Microorganism	Aqueous extract MIC (µg/ml)	Oil MIC (µg/ml)
Gram-negative bacteria		
S. typhi	2560≤	2560≤
E. coli	2560≤	2560≤
P. aeruginosa	2560≤	2560≤
Gram-positive bacteria		
S. aureus	1280≤	1280≤
S. pneumoniae	1280≤	1280≤
S. pyogenes	1280≤	1280≤

Discussion

Conflicting reports have existed about the effects of black seed oil on gram-negative and gram-positive bacteria. In some of them, the effect is more on gram-negative and in another category on gram-positives. Salman *et al*. [38] concluded in their study that oil of *Nigella sativa* and also methanolic extracts were effective against numerous drug-resistant Staphylococcus aureus strains, without cross-resistance having generally authorized antibiotics, and can be utilized minimum topically in sensitive cases. Further research is required to recommend its role in systemic infections.

Hanafy & Hatem [39] showed that microgram concentrations of the ether extract of *Nigella sativa* seeds inhibited the growth of several species of pathogenic bacteria representing Gram-positive bacteria (*Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and a pathogenic yeast (*Candida albicans*). *Salmonella typhimurium* was non-sensitive to the range of concentrations of the extract used in this study. The data suggest that the antibacterial action of the extract may be more pronounced on Gram-positive than on Gram-negative bacteria.

Morsi [18] has determined the anti-microbial effects of the plant extracts in Gram-positive bacteria (*Staphylococcus aureus*), Gram-negative bacteria (*Pseudomonas aeruginosa*, and *Escherichia coli*), and the pathogenic fungus *Candida albicans*.

In a study by Sokmen *et al*. [40], a methanolic extract derived by soxhlet extraction was not active against one strain of *S. aureus* derived from a clinical sample. This may be due to the difference of strains tested.

Al-Ameedy & Omran [41] found that hexane fraction of *Nigella sativa* disclosed a massive antibacterial action against Gram-negative microorganisms, like zone of inhibition was ranging from 18.3mm for both Enterobacter and *Escherichia coli* to 12.6mm for Klebsiella. Since, ethanolic fraction showed more remarkable potential action against *Staphylococcus aureus* with 22.3mm zone of inhibition contrasted to chloroform and hexane fractions, which showed higher impact on Enterobacter, Klebsiella, and *Escherichia coli.* In the same duration, methanol fractions show higher growth activity inhibition against *Staphylococcus aureus* and *Klebsiella pneumonia* with zones of 14.3mm and 10.0±2.9mm, respectively. Each acetone, ethyl acetate, and water fractions did not show any antimicrobial action against test bacteria used in the study. Our study is by these reports.

In a study conducted on rats by Mariam [31], the extracts were shown to have a significant inhibitory effect against gram-positive bacteria, which is in accordance with a part of our findings. However, the extract revealed an equal effect with the standard drug on the clearance of staphylococcal ATCC 29923 infection *in vitro*.

In a study conducted by Rafati *et al*. [42], the antimicrobial effect of oil extract of black seed against two common Staphylococcus aurous in the laboratory was studied. They concluded that the anti-microbial effect of NS oil extract is comparable with antibiotics such as Ceftazidime, Cefaclor, Cefamandole, and Cefuroxime. They recommend the practical use of NS. to control *Staphylococcus aureus* infection.

Zuridah *et al*. [43] investigated *in vitro* antibacterial effects of seed extracts on standard gram-positive and negative bacterial strains. The best result was seen on *S. aurous*. They showed that the methanol extract of *N. sativa* seeds had the best antimicrobial activity to *S. aureus* compared to its activity on the gram-negatives. Although the best effect of seed extract against gram-positive and negative was observed in *E. coli* in our study, we saw a medium effect in *S. aurous*.

Hosseinzadeh *et al*. [44] concluded in their study that seeds of *N. sativa* show effective antibacterial action in both *in vivo* and *in vitro* studies. The results indicated that the chloroform-methanol essential oil extracts effectively against Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) bacteria. The purification and isolation of the active antibacterial plant compound, besides different administration routes of plants, the specifically oral way is suggested. Kamal *et al*. [30] showed that *Nigella sativa* extracts produce antimicrobial activity against many microbes, especially on multiple antibiotic-resistant bacteria. The study's findings are consistent with our study, and we observed the antibacterial activity of *N. sativa*.

Halawani *et al*. [1] demonstrated that thymoquinone and thymohydroquinone had inhibitory and lethal effects against both gram-negative and gram-positive bacteria. When combined with antibiotics, they may exert synergistic activity. Therefore, both thymoquinone and thymohydroquinone could be used as antibacterial drugs.

Fico *et al*. [45] studied the Antimicrobial activity of crude extracts, fractions, and essential oil. The antimicrobial screening was carried out against *Staphylococcus aureus* (ATCC29213) and

Staphylococcus epidermidis (ATCC12228) for the essential oil. The essential oil showed antibacterial activity against *Bacillus cereus* and *Staphylococcus aureus* (Gram-positive bacteria).

Abdallah *et al*. [46], in their work, concluded that scientific literature vast data if referred indicates that black seeds show effectual antimicrobial action against various viruses, bacteria, and fungi and are comparatively safe drug having a long exceptional history in conventional medicine; it also was more powerful than numerous standard antibacterial drugs. It was suggested to develop and design new antibacterial drugs from the seeds of *Nigella sativa*. To do so, the mode of action and mechanisms of the antimicrobial action of black seeds on eukaryotic or prokaryotic cells and viruses should be understood well; applying novel techniques like nanotechnology can help reach the goal.

Conclusion

Nigella sativa has antibacterial activity against gram-positive and gram-negative bacterial isolates.

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