



The Role of *Nigella Sativa* as Anti-Inflammation in *Klebsiella Pneumonia* Infection : A Review

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Abstract

The oil obtained from the seeds of *Nigella sativa* L. (*N. sativa*), also known as black cumin, is frequently used in the Mediterranean area for its anti-inflammatory. The essential oil of *Nigella sativa* (NSO) constituents include p-cymene, thymol, thymoquinone (TQ), dithymoquinone (DTQ) and thymohydroquinone (THQ). *N. sativa* and its main active constituent TQ have been attributed to numerous pharmacological activities. The present study demonstrated the anti-inflammatory effect of NSO in acute inflammation. *Nigella sativa* extract have an effect on very significant to degradation of colony amount of *Klebsiella pneumoniae*. *Nigella sativa* could be potential sources of antimicrobials with protective properties are attributed to reproducible radical scavenging activity as well as an interaction with numerous molecular targets involved in inflammation, including proinflammatory enzymes and cytokines. Furthermore, broad spectrum studies on specific cellular and molecular mechanisms of action as well as controlled clinical trials to prove its efficacy in humans are really needed to further assess the application of *Nigella sativa*.

Keywords: *Nigella sativa*; Anti-inflammation of Bacteria; *Klebsiella Pneumonia*.

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1. Introduction

Oil of *Nigella sativa* revealed effective antibacterial activity against considerable number of methicillin resistant and coagulase negative *Staphylococcus aureus*, safety of that oil was examined, and there was no cytotoxic influence on the proliferation of gingival fibroblasts. The black seed oil was recommended to be used as an antimicrobial agent in food production to prevent spoilage. Based on the results that showed that this oil at 2.0% concentration was able to inhibit the growth of twenty-four pathogenic, spoilage and lactic acid bacteria. Ethanol and n-hexane extracts of the black seeds recorded remarkable dose dependant antibacterial effects against different gram-positive and gram-negative strains, namely *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus epidermidis*, *Klebsiella pneumonia* and *Salmonella typhimurium* [1].

2. *Nigella sativa*

The *Nigella* genus, which belongs to the Ranunculaceae family, is widespread throughout Europe, North Africa, and Asia. In folk medicine, many species of *Nigella* were found to have a significant role. *Nigella sativa* (black seeds) is one of the main plants that is traditionally utilized in folk medicine to cure several diseases, such as stomachic, diuretic, liver tonic, and diaphoretic diseases in different cultures, including Arab and Chinese cultures. Furthermore, *Nigella arvensis* is used locally on cakes and bread as a flavouring agent, as well as a remedy for stomach pains, ulcers, and for diuretic effects. It was reported that *N. sativa* exhibited antioxidant, anti-inflammatory, antimicrobial, anti-carcinogenic and could enhance learning and memory activities [2].

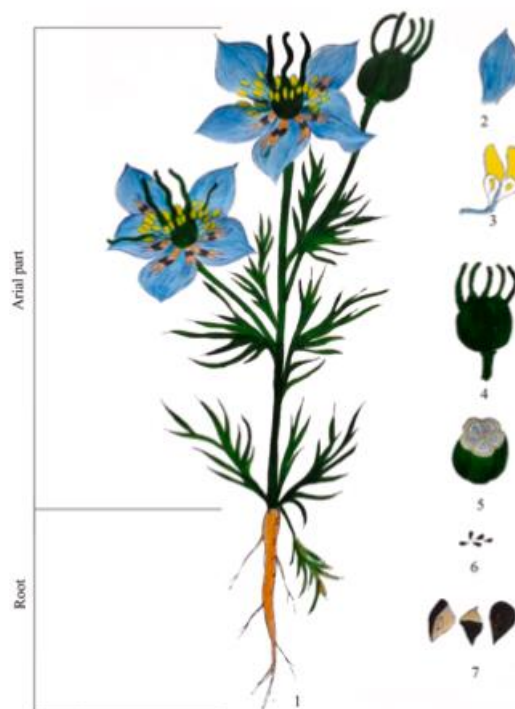


Figure 1: *Nigella sativa* and its different body parts. 1. Habit, 2. Petal, 3. Stamen (front view), 4. Fruit, 5. Fruit (transverse section), 6. Seeds, and 7. Seeds (longitudinal and transverse section) [3]

Nigella sativa seed possesses a large variety of ingredients including fixed oil, essential or volatile oil, proteins, alkaloids and saponins. Oil content of the seed ranges from 30-40%, of which > 98% is fixed and 0.1-2% is essential oil. The major oil components include species of triacylglycerol, saturated and unsaturated fatty acids which may vary according to the extraction method used and the seed's origin. Lesser components include phytosterols, tocopherols and essential oils. The essential oil constituents include p-cymene, thymol, thymoquinone (TQ) (C₁₀H₁₂O₂ or 2-isopropyl-5-methylbenzo-1,4-quinone), dithymoquinone (DTQ) and thymohydroquinone (THQ). TQ is the most abundant (30-50%) bioactive constituent of the seed's essential oils and is thought to provide most of its pharmacological effects [4].

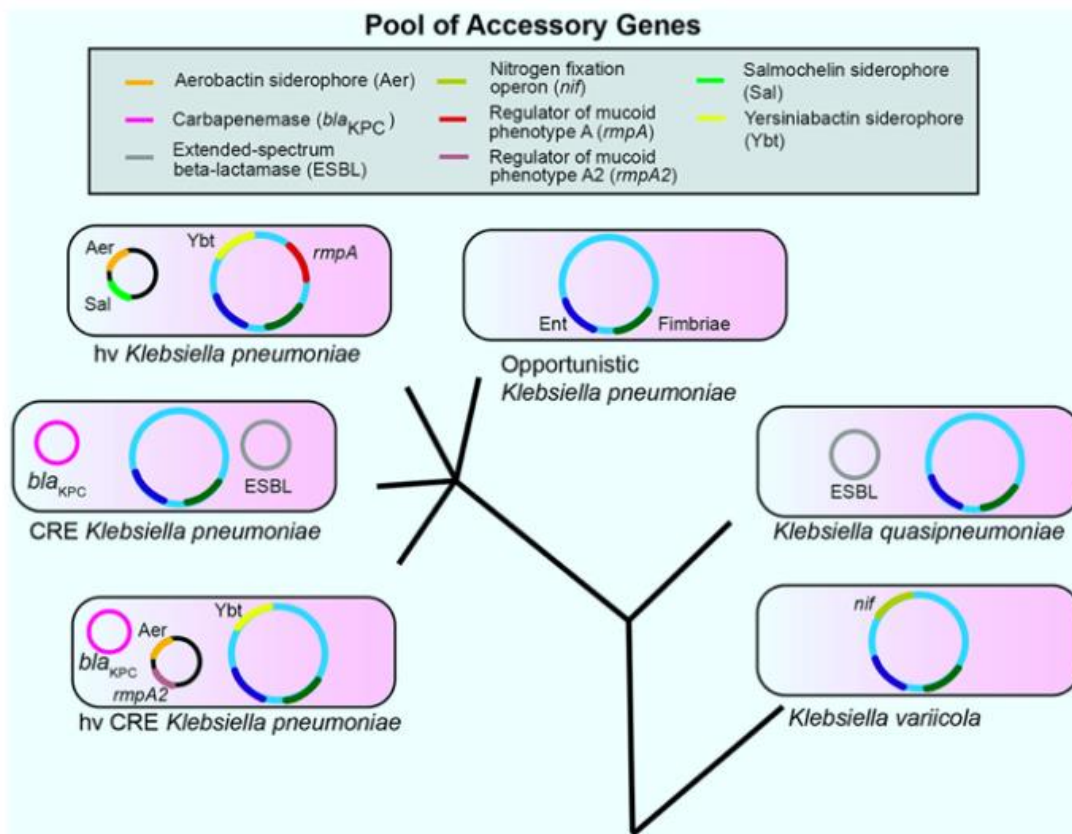


Figure 2: *Klebsiella pneumoniae*, *variicola*, and *quasipneumoniae* are three species that share a pool of accessory genes. The combination of genes in the accessory genome differs between species and pathotypes within *K. pneumoniae* such as opportunistic, carbapenem-resistant (CRE), and hypervirulent (*hv*) strains. These accessory genes can also combine to form new pathotypes (*hvCRE*) and can be shared across species. Enterobactin (Ent, blue) and Fimbriae (dark green) represent conserved genes; accessory genes are shown as examples and are not a definitive list [5]

Thymoquinone (TQ) and thymol concentrations, the main compounds of NSO with an anti-inflammatory effect, were 0.375 (mg/mL) and 0.021 (mg/mL), respectively. The chemical composition of the volatile compounds identified in NSO is presented in **Table 1**. As observed, in the volatile fraction of NSO, 13 compounds were identified, and, among them, p-cymene was the major compound. The monoterpene hydrocarbons group

represented by α -thujene, α -pinene, 4(10)-thujene (sabinene), β -pinene, p-cymene, D-limonene and sabinene hydrate had the highest percentage (62.97%) among identified compounds. The monoterpene ketones, represented by TQ, were the next predominant group, with 29.85%. The other identified compounds were found in a lower percentage (7.18%) as follows: sesquiterpene with 3.72%, terpene phenols with 2.35%, monoterpene alcohols with 0.35% and cycloalkenes (0.76%) [6].

Table 1: Gas chromatography coupled with mass spectroscopy (GS-MS) chemical composition of volatile compounds identified in NSO

Compounds	RT (min)	Concentration (% from Total Peaks Area)
α -Thujene	7.7	12.02
α -Pinene	7.948	2.49
4(10)-Thujene (Sabinene)	9.325	1.02
β -Pinene	9.501	2.39
(+)-4-Carene	10.949	0.76
p-Cymene	11.236	39.72
D-Limonene	11.407	1.61
Sabinene hydrate	14.837	3.72
1-Terpinen-4-ol	17.236	0.35
Thymoquinone	19.806	29.85
Thymol	21.562	2.35
α -Longipinene	23.46	0.66
D-longifolene (Junipene)	25.549	3.05

Abbreviations: min, minutes; RT, retention time.

N. sativa and its main active constituent TQ have been attributed to numerous pharmacological activities. Up to now, cytotoxic, antioxidant, immune enhancement, gastroprotective, hepatoprotective, antitussive, hypolipidemic, and cardioprotective effects, increased milk production, hypoglycemic, hypotensive, and antimicrobial effects have been demonstrated. In addition, beneficial effects of *N. Sativa* and thymoquinone on convulsions, depression, men's infertility, memory improvement, nociception, and inflammation have been discussed [7].

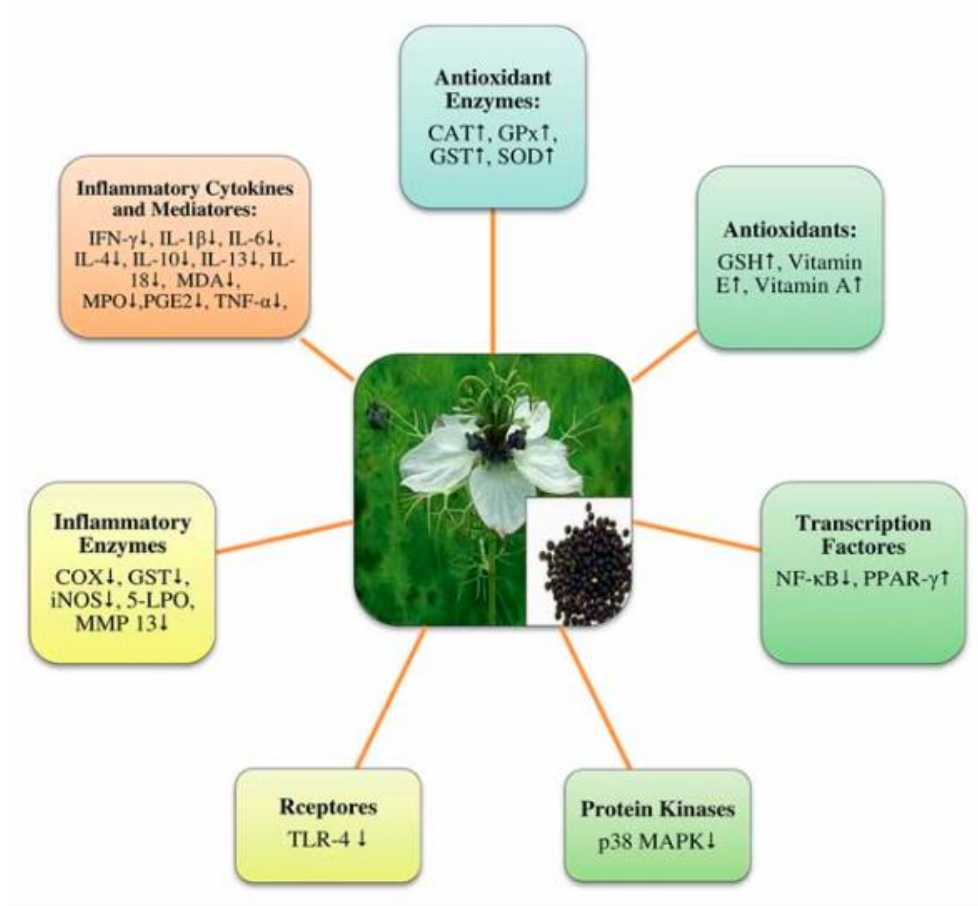


Figure 3: Different molecular targets of *N. sativa* and thymoquinone in inflammatory diseases. CAT: cat- alase; COX: cyclooxygenase; GPx: glutathione per- oxidase; GSH: reduced glutathione; GST: gluta- thione-S- transferase; IFN- γ : interferon gamma; IL: interleukine; iNOS: inducible nitric oxide synthase; 5- LPO: 5- lipooxygenase; p38MAPK: p38 mitogen-ac- tivated protein kinases; MMP: matrix metallopro- teinase; MPO: myeloperoxidase; NF- κ B: nuclear fac- tor-kappa B; PPAR- γ : peroxisome proliferator-acti- vated receptor γ ; SOD: superoxide dismutase; TLR- 4: toll-like receptor; TNF- α : tumor necrosis alpha [7]

3. Klebsiella Pneumonia

Klebsiella pneumonia are gram-negative, encapsulated, nonmotile bacteria that are members of the family Enterobacteriaceae. They can be found in a number of environments, including water and soil, and in association with both plants and animals. In humans, Klebsiella can be found in the intestinal tract; however, certain species, such as *K. pneumoniae*, have been known to cause respiratory and urinary tract infections as well as bloodstream infections. Virulence factors such as capsular polysaccharides, pili, and adhesins are major contributing factors to infections. These factors also play a role in biofilm formation [8].

On agar media, it has a mucoid phenotype that is conferred by the polysaccharide capsule attached to the bacterial outer membrane, and ferments lactose. *K. pneumoniae* is part of the *Enterobacteriaceae* family, which is comprised of other familiar pathogens such as *Escherichia coli*, *Yersinia* species, *Salmonella* species, and *Shigella* species. *K. pneumoniae*, a leading cause of hospital-acquired infections (HAIs) in the United States, has

classically been considered an opportunistic pathogen, since it typically causes infections in hospitalized or otherwise immunocompromised individuals. As the virulence of these bacteria and the demographic features of the patients they infect begin to shift, understanding how *K. pneumoniae* is transmitted and the factors responsible for pathogenicity is important in treating infected patients [5].

K. pneumoniae which is commonly associated with urinary tract infection, pneumonia, bacteraemia and wound infections, is also a primary causative agent of liver abscess particularly in Asia. The strains causing liver abscess are mostly community acquired and associated with a hypermucoid phenotype. These hypermucoviscous types, also called as hypervirulent (hv) *K. pneumoniae* (Kp) are often susceptible to antimicrobials. They have the potential for metastatic spread in young and healthy individuals without a history of hepatobiliary disease [9]. In China, *K. pneumoniae* accounted for 11.9% of isolated pathogens from ventilator-associated pneumonia (VAP) and intensive care unit (ICU)-acquired pneumonia. In addition, carbapenem-resistant Enterobacteriaceae (CRE) caused by *K. pneumoniae* have been reported to account for 73.9% of 664 clinical samples in a multi-center clinical study that covered 25 “AAA” hospitals in 14 provinces of China. There is no doubt that such a high prevalence and mortality rate of *K. pneumoniae* infection caused a great burden on the country’s health system [10].

K. pneumoniae strains usually recognized by microbiologists and clinicians are termed as cKP. Such strains are scandalous for their capability to cause acquired *K. pneumoniae* strains usually recognized by microbiologists and clinicians are termed as cKP. Such strains are scandalous for their capability to cause acquired hospital infections and acquire multidrug resistant especially extended-spectrum beta lactamase (ESBL) that has led the treatment to limited options. ESBLs are plasmid mediated enzymes and it inactivate β -lactam antibiotics such as oxyimino-cephalosporins and oxy-imino-monobactam, except cephamycins and carbapenems [11] and it is inhibited by clavulanic acid and placed it under Bush’s functional class 2be [12].

Hypervirulent *Klebsiella pneumoniae* (hvKp) is an evolving pathotype that poses unique challenges. Classical *K. pneumoniae* (cKp) causes the majority of *Klebsiella* infections in North America and Europe but is less virulent than hvKp as manifested by the ability of hvKp to cause tissue invasive infection in otherwise healthy individuals from the community [13]. To further understand hvKp, the host, pathogen and host–pathogen interactions may be the key element. At present, the prevalence of hvKp in the elderly, especially ESBL-hvKp and MDR-hvKp is increasing. It is essential to enhance the clinical awareness and management of hvKp infections [14]. HvKp was first recognized as a unique clinical entity in the 1980s in Taiwan. Community-dwelling patients, without hepatobiliary risk factors, were presenting with pyogenic liver abscesses caused by *K. pneumoniae*. Doctors and scientists soon discovered that these patients had an invasive type of *K. pneumoniae* with a tendency for metastatic spread to distant sites [15].

Hypervirulent *K. pneumoniae* (hvKp) can be detected in the lungs of Aceh cattle, representing acute and chronic infections. The distribution of *Klebsiella* antigens in the lung tissue was consistent with the histopathological findings [16]. Neutrophil efferocytosis impairment by *Klebsiella pneumoniae* via modulation of cell death pathway, which may provide novel targets for therapeutic intervention of this infection [17].

The current treatments for pneumonia involves the long-term oral administration of high doses of multiple drugs. This treatment can lead to serious side effects including liver or kidney damage. Selectivity of a drug distribution to lungs cannot be measured by an oral route and the difficulty of delivering drugs to side deep within the lungs, to the actual side of infection [18]. The rising incidence of antibiotic resistance in both nosocomial and community-acquired pathogenic bacteria poses a serious threat to global health, compounded by the paucity of new antibiotics in the drug development pipeline and has sparked renewed interest in alternative, non-antibiotic modes of treatment for bacterial infection, including the therapeutic use of bacteriophage, stimulation of immune cellular functions and the development of agent that advantageously modify the antibiotic resistance and virulence of bacterial pathogens [19].

A huge number of studies have demonstrated outbreaks in several hospitals due to the isolation of *K. pneumoniae* resistant to different third-generation antibiotics such as cephalosporins, aminoglycosides and quinolones. The production of antibiotic resistance mechanisms like extended-spectrum β -lactamases and others can be favoured by horizontal transfer of antimicrobial resistance genes using mobile elements like transposons and plasmids, which contribute to *K. pneumoniae* survival in nosocomial environments [20].

4. Nigella sativa anti-inflammation effect in Klebsiella Pneumonia

The volatile oil (0.66 ml and 1.55 mL/kg, i. p.) of *N. sativa* and thymoquinone (0.5, 1.0, 5mg/kg, i.p.) exhibited a dose-dependent anti-inflammatory effect against carrageenan-induced rat hind paw edema and cotton seed pellet granuloma comparable to the reference drug indomethacin (3 mg/kg, i. p.). Similarly, Al-Ghamdi and coworkers demonstrated that the aqueous extract of *N. sativa* possesses an anti-inflammatory action in carrageenan-induced paw edema similar to 100 mg/kg aspirin; however, it had no antipyretic activity on yeast-induced pyrexia. In another study by Hajhashemi and his colleagues as well as Ghannadi and his colleagues both oral and intraperitoneal administration of polyphenols extracted from *N. sativa* as well as essential oil (200, 400, and 800 μ L/kg) containing p-cymene (37.3 %) and thymoquinone (13.7 %) suppressed the early and late phases of the formalin test, acetic acid-induced writhing in mice, carrageenan-induced paw edema, and croton oil-induced ear edema in rats. The authors reported that treatment with naloxone failed to reverse the analgesic activities of both the polyphenols and essential oil. The methanolic extracts of different germination phases of *N. sativa* showed significant anti-inflammatory and antinociceptive effects in kaolin-induced rat paw edema and hot-plate tests, respectively, throughout the duration of the study (1, 3, 6, and 18h after the injection of kaolin). The highest effect was observed from the 5th day to the 11th day of germination [7].

The present study demonstrated the anti-inflammatory effect of NSO in acute inflammation. The anti-inflammatory effects of NSO are comparable with those induced by diclofenac. The analgesic effect of NSO was observed only in the sub-acute inflammation model when the mechanical analgesia was evaluated. In addition, NSO added to diclofenac had a tendency to increase the analgesic and anti-inflammatory effects of diclofenac in sub-acute inflammation, but the differences did not reach the level of statistical significance. The analgetic and anti-inflammatory effects of NSO treatment were related to its antioxidative action, demonstrated by the reduction of MDA and GSSG and increases in DH. The present study offers also valuable information regarding the composition of NS oil. The results of the present study showed that NSO is an important source of

bioactive compounds, especially p-cymene, TQ dan α -thujene. The percentage of the identified compound are consistent with other reported results [6].

The antimicrobial effects of chemically synthesized nano thymoquinone (Tc-NPs) and biologically synthesized nano thymoquinone (Tb-NPs) were evaluated against common antibiotic resistant organisms (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococci faecalis*, *Klebsiella* species and *E. coli*) using agar well diffusion. The results obtained from the UV spectra analysis showed highly intense absorption for both Tb-NPs and Tc-NPs at 242 nm and 319 nm respectively. The FTIR spectrum for Tb-NPs and Tc-NPs showed characteristics peaks at five different regions. The FESEM analysis of Tc-NPs showed micro/nano fibers and were homogeneously distributed and Tb-NPs showed spherical and agglomerates that were unevenly distributed. The cytotoxicity assay showed potential anticancer activity of Tb NPs with minimal concentration that of Tc NPs. Additionally these nTQ particles showed antimicrobial activity with removal of potential of bacterial and fungal pathogens by increased zone of inhibition. The obtained results concluded the biosynthesized nTQ have shown more activity because of the ability to remove potential of microbial pathogens and increase cancer cell death. These findings may encourage the use of biosynthesis method of nTQ over the chemosynthesis method [21].

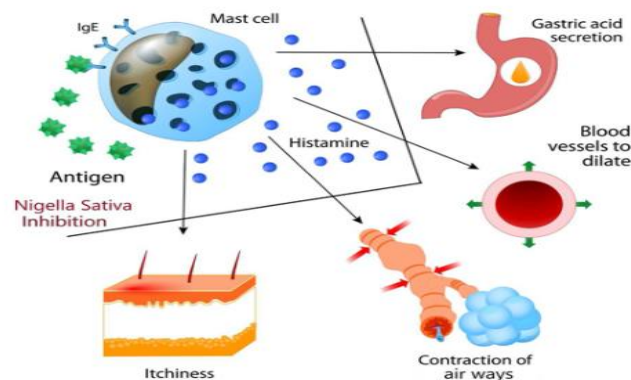


Figure 4: *Nigella sativa* has potential therapeutic action for a diverse range of disease processes including respiratory and dermatological though inhibition of histamine release by mast cells [4]

The reported capability of *N. sativa* to augment the cytotoxic activity of natural killer (NK) cells against cancer cells is also emphasized. The molecular and cellular mechanisms underlying such immunomodulatory and anti-inflammatory effects of *N. sativa* and TQ are highlighted. Moreover, the signal transduction pathways implicated in the immunoregulatory functions of *N. sativa* and TQ are underscored. Experimental evidence suggests that *N. sativa* extracts and TQ can potentially be employed in the development of effective therapeutic agents towards the regulation of immune reactions implicated in various infectious and non-infectious conditions including different types of allergy, autoimmunity, and cancer [22]. *Nigella sativa* extract have an effect on very significant to degradation of colony amount of *Klebsiella pneumoniae*, with got average value of concentration 0% as control that is 1,18 per ml; 50% that is 1 per ml; 60% that is 0,6 per ml; 70% that is 0,4 per ml; 80% that is 0,04 per ml and 90%. Most effective concentration in degrading the amount of colony is 90%,

because in this concentration have not there are colony which grow at media. Therefore cumin (*Sativa nigella*) can be used as determination of bacterium colony amount of *Klebsiella pneumoniae* [23]. Methanol and water extract of the black seed reported remarkable antibacterial efficacy towards *Streptococcus pyogene*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Proteus vulgaris*, the greater antibacterial effect was against the gram-positive bacteria. An active principle isolated from seeds of *Nigella sativa* called thymoquinone showed a broad spectrum of activity against different gram-positive and gramnegative bacteria, namely *Bacillus cereus* ATCC 14579, *Listeria monocytogene* ATCC 19115, *Enterococcus faecalis* ATCC 29212, *Micrococcus luteus* NCIMB 8166, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* CIP 106510, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 35218 *Salmonella enteric*, serovar typhimurium ATCC 14028, *Vibrio liginolyticus* ATCC 33787 and *Vibrio paraheamolyticus* ATCC 17802, thymoquinone was able to prevent bacterial biofilm formation. The potential antibacterial activity of the black seed was also evaluated in-vivo, a groups of male mice were infected with *Staphylococcus aureus* and *Escherichia coli*, and subjected to varied doses of methanol, chloroform and essential oil of the black seed. All extracts and oil revealed significant dose dependant antibacterial effects compared with the positive control group which administered gentamicin [1]. This is a brief review to explore the main structure and activity of *Nigella sativa* which administered to *Klebsiella pneumonia*. A more review is needed that can answer specific questions to explore *nigella sativa* with antimicrobial effect of *klebsiella pneumonia* as well and analysis research biases related to this topic.

5. Conclusion

Nigella sativa could be potential sources of antimicrobials. Many protective properties are attributed to reproducible radical scavenging activity as well as an interaction with numerous molecular targets involved in inflammation, including proinflammatory enzymes and cytokines. This reported remarkable antibacterial efficacy towards *Klebsiella pneumonia*. Furthermore, broad spectrum studies on specific cellular and molecular mechanisms of action as well as controlled clinical trials to prove its efficacy in humans are really needed to further assess the application of *Nigella sativa*.

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6. Conflict of Interest

The author declares that he has no conflict of interest.

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