International Journal for Scientific Research (IJSR)

المجلة الدولية للبحوث العلمية

Vol. (4), No. (1) January 2025 الإصدار (4)، العدد (1)

Study of the inhibitory ability of alcoholic and aqueous extract of Nigella sativa seeds on the growth of staphylococcus aureus bacteria

Faeza Mharb, Hana Faraj
Zoology Department, Faculty of Science, Tobruk University, Tobruk, Libya

Mahboba Aldareh
Foundation Department, Faculty of Medical Technology, Tobruk University, Tobruk, Libya
mahboba.aldareh@tu.edu.ly

Abstract

Staphylococcus aureus (S. aureus) stands as a prominent pathogen in both hospital and community settings, causing a spectrum of infectious diseases. These range from mild skin and soft tissue infections to more severe conditions like infective endocarditis, osteomyelitis, bacteraemia, and potentially fatal pneumonia. The escalating drug resistance of S. aureus, driven by bacterial evolution and antibiotic misuse, has become a pressing concern in healthcare. Nigella sativa (N. sativa), a member of the Ranunculaceae family, is an herb indigenous to regions spanning the Middle East, Europe, and Western and Middle Asia. This plant has a long-standing history of use as a nutritional supplement and therapeutic agent for various infections and chronic conditions. Given S. aureus's remarkable adaptability to antibiotic pressure, which poses a significant health challenge, the exploration of novel antimicrobial agents has become imperative. A research initiative at the University of Tobruk sought to investigate the antibacterial efficacy of N. sativa seeds against S. aureus. The study employed the cup-plate agar diffusion technique under controlled laboratory conditions. Results indicated that while aqueous extracts of N.

sativa seeds failed to inhibit S. aureus growth, the alcoholic extract demonstrated clear inhibitory effects, even at the lowest tested concentration. These findings corroborate the antibacterial properties of N. sativa seeds and provide scientific backing for the plant's traditional application in treating bacterial

Keywords: Nigella, N. Sativa, Black Seed, S. Aureus, Tobruk University.

Introduction

The genus Nigella is relatively small and contains about 18 species with several sub-species and several genotypes. All Nigella species are therophytes (annuals); that mean complete their life cycle in a short favourable time and survive harsh periods as seeds. The name N. sativa comes from the Latin word, nigellus, meaning black, it is also known around the world by many other names because of its ancient popular history and medicinal value. The Arabic name of N. sativa is 'Al-Habbatus Sauda' mean black seed or 'Habbat al Baraka' that mean the seed of pleasing, while in old Latin it was called "Panacea" meaning "cure all" (Hussain and Hussain, 2016). The common English name for N. sativa is Love in a Mist. It is known Kalijeera" (Bangladesh), "Kalonji" (in India) and "Hak Jung Chou" (China), carvi (French), schwarzkummel (German), kalonji (Hindi/Urdu), kezah (Hebrew) chernushka (Russian), corek-out (Turkish), siyahdaneh (Abdallah, 2017). N. sativa, perhaps the most well-known member of the genus Nigella, it is about 8-35 inches (20-90 cm) in height and has finely divided, somewhat threadlike leaves. This species has pale-blue to pale-purple flowers that flourish in the spring and produce seed capsules (fruit) that contain many black seeds (Engels and Brinckmann, 2017). Nigella sativa, is annual flowering plant, it is grown in many parts of the world (Abdallah, 2017). N. sativa is found growing wild in regions of northern Africa, Turkey, Syria, Iraq, and Iran. The species is also cultivated on a commercial scale in northern Africa (Egypt, Tunisia, Sudan), eastern Africa (Ethiopia), western Asia (Iraq, Jordan, Lebanon,

Syria, Turkey, Yemen), and southern Asia (India, Iran, Pakistan) (Engels and Brinckmann, 2017).

The traditional uses of Nigella sativa one of the earliest cultivated plants in human history. Originating from ancient Egypt, Greeks and the Romans (Al-Jaafary et al., 2016). The black seeds have been prescribed by ancient Egyptian and Greek physicians to treat headache, nasal congestion, toothache and intestinal worm, as well as a diuretic to promote menstruation and milk production (Khan et al., 2011). The seed and oil of N. sativa was commonly used in ancient remedies in Unan, Ayurveda, China, Middle-East, Arabic and Asian countries (Islam et al., 2017). They have been reported to be used in folk traditional medicine as remedy for asthma, hypertension, diabetes and cough (Emeka et al., 2015). Nigella sativa seeds contain fixed oil, proteins, alkaloid, saponin and essential oil. It is composed of unsaturated fatty acid that includes (arachidonic, eicosadienoic, linoleic and linolenic acid). The saturated fatty acid present in the oil is (palmitic, stearic and myristic acid). The pharmacologically active constituents of volatile oil are (thymoquinone, dithymoquinone, thymol and thymohydroquinone) (Khan et al., 2016). Interestingly, these tiny seeds are rich in bioactive compounds of pharmacological benefits are nigellicine, nigellicimine, nigellicimine N-oxide, carvone, thymoquinone, thymol and many more (Parrakah, 2010).

Different crude extracts of N. sativa exhibited effective antimicrobial activity against different bacterial strains either Gram negative or Gram-positive bacteria. The most effective extracts of N. sativa were the crude alkaloid and water extracts. Gram-negative isolates were more susceptible than the Gram-positive ones (Sultana et al., 2015).

Thymoquinone, Thymol and other active compounds the Black seed contribute to have its medicinal properties (Thilakarathna et al., 2018). Black seed's antimicrobial activity effect on Gram-negative and Gram-positive bacteria, viruses, parasites,

Schistosoma, and fungi pathogens (Tavakkoli et al., 2017). The antimicrobial activities of thymoquinone were determined against different bacterial strains. Among sixteen oral strains, seven strains including S. aureus were found to be sensitive to thymoquinone (Nasir and Saba, 2015). Methanol and water extract of Nigella sativa reported to have effective antibacterial activity towards Streptococcus pyogenes, Pseudomonas aeruginosa, Klebsiella pneumonia, and Proteus vulgaris, the greater antibacterial effect was against the Gram-positive bacteria (Hasan et al., 2013, Abdallah 2017). N. sativa has been reported to exhibit many pharmacological properties that include antimicrobial action against bacteria (Khan et al., 2013). N. sativa oil as well as methanolic extract are active against multidrug resistant S. aureus strains (Salman et al., 2016). Also, petroleum ether extract of N. sativa has been reported to possess antimicrobial activity against S. aureus and some pathogenic clinical isolates (Ali, 2015). Aqueous extract of N. sativa was possessing bacteriostatic activity against S. aureus and some clinical isolates; were observed as compared with control aqueous extract can slow down the multiplication of the selected clinical isolates after overnight incubation (Anjum et al., 2015). This study conducted at the University of Tobruk aimed to evaluate the antibacterial properties of N. sativa seeds against S. aureus.

Material and Method

First: Sterilization:

Ready and sterilized culture media were obtained from Ibn Rushd Laboratory. As for the glassware, it was sterilized in the hot oven at 180 C^0 after washing it with distilled water. Alcohol was used to sterilize the work table, and the work was done near the flame**.**

International Journal for Scientific Research, London Vol (4), No (1), 2025
https://doi.org/10.59992/IJSR.2025.v4n1p2 E-ISSN 2755-3418

Obtaining and Processing Nigella Sativa Seeds (Extraction Method):

Nigella sativa seeds were obtained from a store in the city of Tobruk, and they were washed, dried in the oven, and ground:

- **Alcoholic extract:** 10 grams of the powder were taken and 100 ml of ethyl alcohol with a concentration of 80% was added and left for a day, then filtered and the resulting solution was taken and used in the inhibition process
- **Aqueous extract:** 20 grams of black seed powder were taken, 100 may of distilled water was added, and the mixture was mixed with the help of heat, using a magnetic heater. After melting, it was left for a day, then filtered, and the resulting solution was taken and used in the inhibition process.

Determination of Zone of Inhibition

0.5ml of various plant extracts of 100 mg/ml were transferred into each well with diameter of 6 mm by a pipette to culture media with bacteria. The plates were incubated at 37 °C for 18 to 24 hrs. The antimicrobial activity was evaluated by measuring the diameters of zone inhibition surrounding the crude extracts. The zones of inhibition were measured in millimetre. The assay was performed by agar well diffusion method for determination of inhibition zone and broth microdilution method.

Result

- **The Effect of the Aqueous Extract of Nigella Sativa:**

  The plates were placed in the incubator after culturing them with the tested bacteria at a temperature of 37 C^0 for 24 hours, then the inhibition zone was recorded on the basis of (+) effective: (-) no effective as shown in the table below.

  The results show, there is a clear growth of bacteria in the area of inhibition.

International Journal for Scientific Research, London Vol (4), No (1), 2025
https://doi.org/10.59992/IJSR.2025.v4n1p2 E-ISSN 2755-3418

(Table .1): Inhibition efficacy of the aqueous extract of Nigella sativa seeds on the growth of the tested bacteria

tested bacteria	effect of aqueous extract
Staphylococcus	-

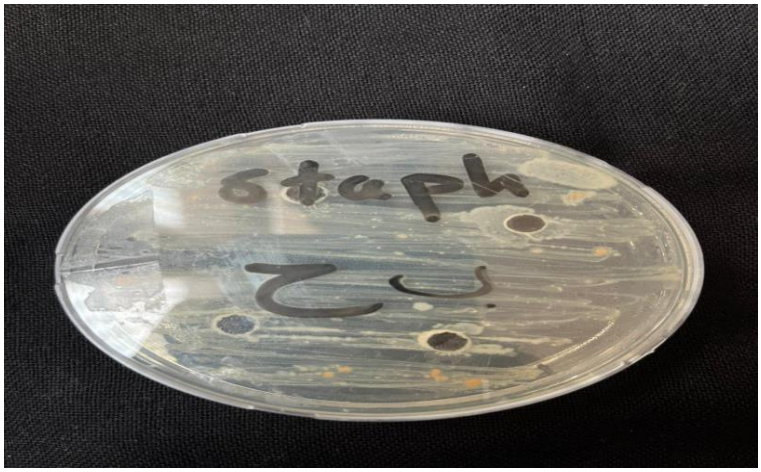

Figure (1): The inhibition zone of Staphylococcus aureus

- **The effect of the alcoholic extract of Nigella sativa:**

The plates were placed in the incubator after culturing them with the tested bacteria at a temperature of 37 C^0 for 24 hours, then the inhibition zone was recorded on the basis of (+) effective: (-) no effective as shown in the table below.

The results show significant effect in inhibition zone by mean (112.5mm)

(Table .2): Inhibition efficacy of the alcoholic extract of Nigella sativa seeds on the growth of Staphylococcus

tested bacteria	effect of aqueous extract
Staphylococcus	+

Figure (2): The inhibition zone of the alcoholic extract of Nigella sativa on Staphylococcus aureus

Discussion

The importance of medicinal plants and herbs in treating some diseases is increasing in our local and Arab societies as a whole. Among the most important of these medicinal plants is the use of Nigella sativa, which was used to treat various diseases.

The use of these plants is common in many countries, including Libya, because some believe that they contain effective compounds and antimicrobial substances. There is no doubt that studying medicinal plants and determining the effectiveness of inhibiting germs is an important scientific method to open the way for benefiting from these natural plants.

Nigella sativa has been used since ancient times as nutritional supplement and for the treatment of various infections and chronic ailments. Staphylococcus aureus is one of the important resistant pathogenic bacteria extremely adaptable to antibiotics pressure. It becomes a major health problem; so that use of new antimicrobial agents is the best choice to overcome this problem (Alshareef, 2019).

In this research, the effect of aqueous and alcoholic extracts of Nigella sativa seeds on a pathogenic gram-positive bacteria was studied .Through the results of this study, it was proved that the aqueous extract of black seed powder did not have any

inhibitory effect on the growth of bacteria used in this study for unspecified reasons, but it can be assumed that the active substances concentrated in the nigella sativa need to be liberated for effect, and this may be confirmed by the strong effect of the alcoholic extract on the growth of Staphylococcus tested. Or the concentration of the active substance is less than the concentration affecting growth.

And by comparing this study with the results of the study of Salman (2005), it was found that there is agreement in terms of influence S. aureus organic extract on the growth of bacteria at the same concentration of 10% used for both studies (Salman, 2005; Al-Qasim, 2016).

These results agreed with study of (Anjum et al., 2015) showed that methanolic extract have activity against S. aureus with maximum zone of inhibition (22.3 mm). Variation in MIC and zone of inhibition may be due to the variation in the nature and combination of phytocompounds present in extract due to environment or type of soil. The results disagree with (Kakil, 2013; Ali, 2015) who found that methanolic extract showed no antibacterial activity. This variation may be due to the difference in method of extraction that used.

This result disagreed with Study (Alshareef, 2019)revealed that aqueous extract of N.sativa performance in inhibition of bacterial growth, the maximum inhibition zone at high concentration was observed (20 mm) and MIC 25 mg\ml. and also disagreed This result with (Khan et al., 2013), who found that aqueous extract of N.sativa has activity against S.aureus with maximum zone of inhibition (21 mm). Also disagreed with study of (Anjum et al., 2015) with maximum zone of inhibition (15 mm). While the study agreed with (Ramli et al., 2014). They report that aqueous extract has no activity against S. aureus. The variation in the results may be due to the variation in the method of antibacterial activity, extraction method and the difference of environment and soil. More research work is required to validate these results and to

determine the role of the other remaining compounds of N. Sativa in antimicrobial activity using advanced techniques.

Conclusion

In our local communities and across Arab societies, there is a growing recognition of the value of herbal and medicinal plants in treating various ailments. One particularly significant example of these therapeutic botanicals is Nigella sativa, which has been employed to address a range of medical condition. Additional studies are necessary to confirm these findings and explore the antimicrobial properties of N. sativa's other remaining components using sophisticated methodologies.

References

- Abdalmoneim, M., Elniema, I. E., & Gumaa, S. A. (2002). Antibacterial activity of Nigella sativa linn. Journal of Science and Technology, 3 (2), 78-86.
- Abdurahim, M. M. (2015). Antibacterial activity of Green tea extract against Staphylococcus nasal carriage among health care workers in Omdurman teaching hospital. Khartoum-Sudan. M.Sc. Thesis. Sudan University for Science and Technology.
- Abdallah, E. M. (2017). Black seed (Nigella sativa) as antimicrobial drug: A Mini-Review. Novel Approaches In Drug Designing Development, 3 (2), 1-4.
- Akmatov, M. K., Mehraj, J., Gatzemeier, A., Stro¨mpl, J., Witte, W., Krause, G., Pessler, F. (2010). Serial home-based self-collection of anterior nasal swabs to detect Staphylococcus aureus carriage in arandomized population-based study in Germany. International Journal of Infectious Disease, 25, 4-10.
- Ali, M. E. (2015). Antibacterial activity of Nigella sativa extract against selected bacterial clinical isolates from Khartoum state. Sundan-khartoum. M.Sc. Thesis. Sudan University of science and technology.
- Al-Jaafary, M., Al-Atiyah, F., Al-Khamis Ebtesam, E., Al-Sultan, A., & Badger, E. L. (2016). In-vitro studies on the effect of Nigella sativa Linn., seed oil extract on Multidrug resistant Gram positive and Gram negative bacteria. Journal of Medicinal Plants Studies, 4 (2), 195-199.

- Alshareef, Inas Adil Ali. (2019). Antimicrobial Activity of Nigella sativa Seeds Extracts against Staphylococcus aureus Nasal Isolates among Sudan University of Science and Technology Students. Sudan University of Science & Technology.
- Anjum, K., Abbas, T., Kazim, M. R., Subhan, S. A., Abdul Wahab, Mehmood, N., Mujahid, T., Hasan, N. (2015). Antimicrobial activity of Nigella sativa aqueous extract, methanolic extract and oil of clinical isolates. International Journal of Current Research, 7 (2), 12294-12299.
- Ayoub, A.O. (2015). Biological activities and some physiochemical characteristics of the Black cumin (Nigella sativa) seed oil. M. Sc. T thesis .Al-Gezira, Sudan: University of Gezira- faculty of engineering and technology.
- Behzad, M.N., Akhi, M.T., Alizadeh, M., Saleh, P., Jafarzadeh, S., Navi, Z.S., Bagheri, M.M., Barband, S., Sadeghi, G., Asghari, B. (2015). Staphylococcus aureus: resistance pattern and risk factors. Journal of Analytical Research in Clinical Medicine, 3 (1), 43-50.
- Benlafya, K., Karrouchi, K., Charkaoui, Y., El Karbane, M., & Ramli, Y. (2014). Antimicrobial activity of aqueous, ethanolic, methanolic, cyclohexanic extracts and essential oil of Nigella sativa seeds. Journal of Chemical and Pharmaceutical Research 6 (8), 9-11.
- Baos Muñoz, Elvira. (2016). Caracterización y seguimiento de la resistencia a linezolid en staphylococcus epidermidis en la unidad de cuidados intensivos del Hospital Clínico San Carlos tras la descripción del primer brote de staphylococcus aureus linezolid resistente.
- David, M. Z., & Daum, R. S. (2010). Community-associated methicillin-resistant Staphylococcus aureus. Clinical Microbiolgy Reviews, 23, 616–687.
- Engels, G., & Brinckmann, J. (2017). Nigella - Nigella sativa. Journal of the American Botonical council, 8 (114), 8-16.
- Emeka, L. B., Emeka, P. M., & Khan, T. M. (2015). Antimicrobial activity of Nigella sativa L. seed oil against multi-drug resistant Staphylococcus aureus isolated from diabetic wounds. Paistan of Pharmceutical Science., 28 (6), 1985-1990.
- Fadailallah, A. F., Mahgoub, A., & Hamid, M. E. (2012). In vitro sensetivity of some mycolic acid-containing Actinomycetes to Nigella stiva extracts. American Journal of Medicine and Medical Science, 2 (6), 111-113.
- Guo, Yunlei, Song, Guanghui, Sun, Meiling, Wang, Juan, & Wang, Yi. (2020). Prevalence and therapies of antibiotic-resistance in Staphylococcus aureus. Frontiers in cellular and infection microbiology, 10, 107.

- Hasan, N. A., Nawahwi, M. Z., & Ab Malek, H. l. (2013). Antimicrobial Activity of Nigella sativa Seed Extract. Sains Malaysiana, 42 (2), 143–147.
- Hussain, D. & Hussain, M.M. (2016). Nigella sativa (black seed) is an effective herbal remedy for every disease except death – a Prophetic statement which modern scientists confirm unanimously: A review. Advancement in Medicinal Plant Research, 4(2), pp. 27-57.
- Islam, M. T., Riaz, T. A., Shahadat, S., Sousa, L. d., Shahadat, S., Sousa, L. d., Leonardo .R., Santos, J. V., Junior, J. J., de Lima, R. M., Baraga, A. L., dos Reis, A.C., Barros, A., Marcus V.O., Melo-Cavalcante, A. (2017). Nigellalogy: A review on Nigella sativa. MOJ Bioequivalence & Bioavailability, 3 (6), 1-15.
- Kabbashi, S.A., Garbi, M., E. Osman, E., M.Dahab, M., S.Koko, W., & Abuzaid, N. (2015). In vitro antimicrobial activity of ethanolic seed extract of Nigella sativa (Linn) in Sudan. African Journal of Microbiological Research, 9 (11), 788-792.
- Kakil, S. (2013). Antibacterial activity of Zingiber officinal, Matricaria chamomilla and Nigella sativa extracts on growth of pathogenic bacteria isolated from different clinical specimens. Khartoum, Sudan. M.Sc. Thesis. Sudan University of Science and Technology.
- Khan, A. M., Qureshi, R.A., Gillani, S.A., & Ullah, F. (2011). Antimicrobial activity of selected medicinal plants of Margalla hills. Journal of Medicinal Plants Research, 5 (18), 4665-4670.
- Khan, A. U., Ali, S., Ur Rehman, A., Ali, H., Ahmed, T., Wagar, M., Niaz, Z. (2013). Antibacterial activity of Nigella sativa and Piper nigrum. Asian Journal for Natural and Apllied Science, 2 (4), 173-179.
- Khan, Z., Hasan, N., Ahmad, N., & Khalid, M. (2016). Pharmacological Activity of Nigella sativa: Areview. World Journal of Pharmaceutical Sciences, 4 (5), 234-241.
- Khoshnood, S., Heidary, M., Asadi, A., Soleimani, S., Motahar, M., Savari, M., et al. (2019). A review on mechanism of action, resistance, synergism, and clinical implications of mupirocin against Staphylococcus aureus. Biomed. Pharmacother. 109, 1809–1818. doi: 10.1016/j.biopha.2018.10.131
- Klein, E. Y., Mojica, N., Jiang, W., Cosgrove, S. E., Septimus, E., Morgan, D. J., et al. (2017). Trends in methicillin-resistant Staphylococcus aureus hospitalizations in the United States, 2010–2014. Clin. Infect. Dis. 65, 1921–1923. doi: 10.1093/cid/cix640
- Klevens, R. M., Morrison, M. A., Nadle, J., Petit, S., Gershman, K., Ray, S., et al. (2007). Invasive methicillin-resistant Staphylococcus aureus infections in the United States. JAMA 298, 1763–1771. doi: 10.1001/jama.298.15.1763.

- Lakhundi, S., and Zhang, K. (2018). Methicillin-Resistant Staphylococcus aureus: molecular characterization, evolution, and epidemiology. Clin. Microbiol. Rev. 31: e00020–18. doi: 10.1128/CMR.00020-18.
- Lessa, F. C., Mu, Y., Ray, S.M., Dumyati, G., Bulens, S., Gorwitz, R. J., et al. (2012). Impact of USA300 methicillin-resistant Staphylococcus aureus on clinical outcomes of patients with pneumonia or central line-associated bloodstream infections. Clin. Infect. Dis. 55, 232–241. doi: 10.1093/cid/cis408.
- Lindsay, J. A. (2013). Hospital-associated MRSA and antibiotic resistance-what have we learned from genomics? Int. J. Med. Microbiol. 303, 318–323. doi: 10.1016/j.ijmm.2013.02.005.
- Mediavilla, J. R., Chen, L., Mathema, B., and Kreiswirth, B. N. (2012). Global epidemiology of community-associated methicillin resistant Staphylococcus aureus (CA-MRSA). Curr. Opin. Microbiol. 15, 588–595. doi: 10.1016/j.mib.2012.08.003
- Mohammed, H. A. (2015). Antibacterial activity of Lawsonia inermis (Sudaneese Henna) leaves extracts against Staphylococcus aureus, Esherichia coli and Pseudomonas aeruginosa among recurrent urinary tract infection patients in Omdurman millitary hospital. Khartoum, Sudan. M.Sc. Thesis. Sudan University of Science and Technology.
- Nasir, U., & Saba, I. (2015). Evaluation of the antimicrobial activity of aqueous, ethanolic and methanolic extracts of Nigella sativa (kalonji) against Gram positive and Gram negative bacterial isolates. Paistank Journal of Biochemistry and Moecular Biology, 48 (4), 92-96.
- Onyeagwara, N., Obasuy, O. i., Ottih, I., & O. Akerele, J. (2014). Nasal carriage of methicillin-resistant Staphylococcus aureus among hospitalized otorhinolaryngological patients in Benin City of Nigeria. Archives of International Surgery, 4 (2), 99-103.
- Ouidri, M. A. (2018). Screening of nasal carriage of methicillin-resistant Staphylococcus aureus during admission of patients to Frantz Fanon Hospital. New Microbes and New Infections, 23, 52–60.
- Otto, M. (2013). Community-associated MRSA: what makes them special? Int. J. Med. Microbiol. 303:324–330. doi: 10.1016/j.ijmm.2013.02.007.
- Parrakah, P. M. (2010). Nigella sativa - Acomperhensive review. Indian journal for natural Products and resources, 1 (4), 409-429.

- Pichereau, S., and Rose, W. E. (2010). Invasive community-associated MRSA infections: epidemiology and antimicrobial management. Expert Opin. Pharmacother. 11, 3009–3025. doi: 10.1517/14656566.2010.511614.
- Ramli, Y., Karrouchi, K., Benlafya, K., Charkoui, K., & Alkarbane, M. (2014). Antibacterial activity of aqueous, methanolic, ethanolic, cyclohexanic extract and essential oil of Nigella sativa extract. Journal of chemical and pharmaceutical research, 6 (8), 9-11.
- Rayner, C., and Munckhof, W. J. (2005). Antibiotics currently used in the treatment of infections caused by Staphylococcus aureus. Intern. Med. J. 35(Suppl 2):S3–16. doi: 10.1111/j.1444-0903.2005.00976.x.
- Rao, Q., Weilong, S., Xiaomei, H., and Xiancai, R. (2015). "Staphylococcus aureus ST121: a globally disseminated hypervirulent clone. Journal of Medical Microbiology, 64, 1462–1473.
- Salas, D. E., Minejima, E., Joanna.W., Fang, C., Wang, J., Rosemary,S., Nieberg, P., Wong-Beringer, A. (2017). Staphylococcus aureus bacteremia in patients not meeting sepsis criteria:clinical features, host immune response and outcomes. Journal of Clinical Medicine and Therapeutics, 2(4), 1-7
- Salman, M. T., Khan, R. A., & Shukla, I. (2005). Antimicrobial activity of Nigella sativa oil against Staphylococcus aureus obtained from clinical specimens.
- Salman, M. T., Khan, R. A., & Shukla, I. (2016). Antibacterial activity of Nigella sativa Linn. Seeds against multiple antibiotics resistant clinical strains of Staphylococcus aureus. International Archives of Bio Medical and Clinical Research, 2 (3), 96-99.
- Schulte, R. H., and Munson, E. (2019). Staphylococcus aureus resistance patterns in Wisconsin: 2018 surveillance of Wisconsin organisms for trends in antimicrobial resistance and epidemiology (SWOTARE) program report. Clin. Med. Res. 17, 72–81. doi: 10.3121/cmr.2019.1503.
- Shang, W., Hu, Q., Yuan, W., Cheng, H., Yang, J., Hu, Z., et al. (2016). Comparative fitness and determinants for the characteristic drug resistance of ST239-MRSAIII- t030 and ST239-MRSA-III-t037 strains isolated in China. Microb. Drug. Resist. 22, 185–192. doi: 10.1089/mdr.2015.0226
- Stark, L. (2013). Staphylococcus aureus - aspects of pathogenesis and molecular epidemiology P.78. In http:// urn.kb.se/resolve?urn=urn :nbn:liu:diva-97343 .
- Sultana, S., Asif, H., Akhtar, N., Iqbal, A., Nazar, H. and Rehman, R., (2015).Nigella sativa: Monograph. Journal of Pharmacognosy and Phytochemistry, 4(4), pp.103-106.

- Tavakkoli, A., Mahdian, V., Razavi, B. M., & Hosseinzadeh, H. (2017). Review on clinical trials of Black seed (Nigella sativa) and its active constituent, Thymoquinone. Journal of Pharmacopuncture, 20 (3), 179-193.
- Thilakarathna, R., Mudhusankha, G., & Navaratne, S. (2018). Phytochemical analysis of Indian and Ethiopian Black cumin seeds (Nigella Sativa). Agricultural Research and Technology, 17 (1).