Investigating the Efficiency of Simple Aqueous Extract of Nigella Sativa Activity Against Serratia Marcescens Bactria

Samir Laybi Shkhaier¹, Muthana A. Majid ¹*, Wathiq Mohammed Allawi², Jamal Abed Al-Fatah¹, Hayder Hamed Abed¹

¹ Basic Sciences Department, College of Dentistry, Mustansiriyah University, Baghdad, IRAQ
² Department of Pharmacy, Al-Rafidain University College, Baghdad, IRAQ

*Correspondent contact: muthana.uomscr@gmail.com

ABSTRACT

Nigella sativa seeds is considered as a very effective treatment for a variety of human diseases in traditional medicine worldwide with low human toxicity. Serratia marcescens pathogenic species, a Gram-negative, opportunistic bacteria, are widespread and distributed in the human environment with considered resistance to different types of antibiotics. Extraction was performed on 12.5 gm./150 ml of Nigella seeds using rotary evaporator. Different time periods for extraction were applied (1-4 hours) with different temperature ranges of 50 °C to 60 °C performed separately for each time period. The 3 and 4 hours extraction period at 60 °C for seeds was effective against Serratia marcescens with inhibition zone of 11.18 and 12.27 mm respectively. Nigella seeds could be a promising inhibitor with enhanced activity against Serratia marcescens. Water extraction was efficient and effective at 60 °C, with extraction period of 2-3 hours for seeds. No activity recorded below this temperature.

KEYWORDS: Nigella Sativa; Serratia marcescens; water Nigella Extract activity; Treatment of Serratia marcescens with nigella sativa.

INTRODUCTION

Seeds of Nigella sativa or black cumin, commonly known as black seed, have been traditionally used in treatment of headache, abdominal pain (i.e. colon, pancreatic, liver, lung, fibrosarcoma, and prostate tumor), coughs diarrhea, lipedema, asthma, rheumatism and other different diseases [1,2]. The seeds of this plant extracts are well investigated, both aqueous and oil extraction methods and have shown antioxidant, anti-inflammatory, anticancer, analgesic as well as antimicrobial activities [3-5]. Administration of Nigella sativa extract was safe and had no notable side effects on liver, kidneys, or gastrointestinal tract [6,7]. Qidwai et al. and others recorded that the administration of Nigella sativa seeds have no effect on serum enzyme, alanine aminotransferase (ALT), and the serum creatinine (Cr) concentration with potential treatment effect [8-10]. Different herbal extraction techniques were used (purification and fractionation) for effective collection of metabolic products. However, extraction conditions (temperature, solvents, agitation speed etc.) may enhance extraction of active ingredients [11]. Serratia marcescens pathogenic species are opportunistic Gram-negative bacteria also considered as tribe Klebsielleae [12]. It is widely distributed in the environment and can pose a significant problem when found in the respiratory, digestive, and urinary tracts in human. Healthcare workers, as well as other individuals are at risk of exposure to Serratia marcescens [13]. Intensive care units (ICUs) at hospitals can develop epidemics of infection with these bacteria since it is found in medical and laboratory equipment,
medications, blood products, antiseptics solution, lotions, and toilets [14,15]. Infections occurring by *Serratia marcescens* are difficult to cure. This difficulty might be as a result of its attribution to resistance to different antibiotics, including ampicillin and first and second generation's cephalosporin's [16,17]. Aminoglycosides provided good activity against *Serratia marcescens*, but resistant strains have also been reported recently by researchers [18]. Other researchers reported an endocarditis caused by a ciprofloxacin-resistant strain of *Serratia marcescens* in which was isolated from blood culture taken from a peripheral vein and the Hickman line [19,20]. The aim of this research is to evaluate the biological activity of a simple aqueous extract of *Nigella sativa* on *Serratia marcescens* bacteria. Moreover, investigate the minimum temperature and time to collect potential aqueous extract of *Nigella* seeds. This extract could provide a powerful treatment against *Serratia marcescens* bacteria with low cost extraction methods. To the time of writing this manuscript, no similar studies focus on the biological ability of *Nigella sativa* aqueous extract on *Serratia marcescens*.

**MATERIALS AND METHODS**

All the glassware had been washed with distilled and deionized water and dried using a hot air oven before use. Mueller Hinton Agar was obtained from HiCrome™ Bulgaria [21, 22]. Seeds of *Nigella sativa* were purchased from commercial market. All other chemicals and reagents used in this research are with high purity for analytical purposes.

**Extraction of Nigella sativa seeds**

12.5 gm of well washed seed samples were extracted in 150 ml of water using rotary evaporator at 50 °C and 60 °C separately for periods 1, 2, 3 and 4 hours, respectively for each selected temperature.

**UV-Vis Spectra Analysis**

The extract was well filtered; scanning was performed by using shimadzu UV-VIS spectrophotometer (UV-1650 pc) with scanning ranged from 200-700 nm.

**Antibacterial Assay**

*Serratia marcescens* bacteria were grown in Mueller Hinton Agar. The bacteria were inoculated in the LB medium in an incubator thermostat for 6 h at 37°C. One milliliter of bacterial inoculum was added to 9 mL 0.9 % normal saline and diluted to 10^6 cfu mL⁻¹ (colony forming unit, cfu), then inoculated into LB broth for 24 hour at 37°C.[23] Well agar diffusion method was used to detect the inhibition zones in sterile molten Mueller Hilton agar with *Nigella sativa* extract. Then plates were incubated at 37 °C for 24 h with the different prepared concentrations of *Nigella sativa* extraction conditions [24, 25]. The aqueous extraction in this work was performed in relatively low temperature to avoid overheating or decomposition of organic compounds during the extraction process. Nevertheless, this work was designed to apply aqueous extraction to avoid any toxicity, interference of solvents, simplicity, and other complications of organic solvents [26].

**RESULTS AND DISCUSSION**

Aqueous herbal extraction one of the most familiar methods for medicinal herbal active ingredients administration. The collected results predicted a biological activity of extracted solution on *Serratia marcescens* bacteria with diameters of inhibition zone 11.18 and 12.27 mm by applying extractions condition of three to four hours respectively at 60 °C. The inhibition zone is represented in Figure 1. However, lowering of extraction temperature and time periods eliminates its biological activity on *Serratia marcescens* bacteria. The results shows powerful effects of *Nigella sativa* seeds watery extract on bacteria without reach a drastic or complex biochemical extraction process [27-29].

The UV-VIS spectrum reported an increase of extracted compounds with increased temperature. The extract spectrum shows an overlap of detection peaks which could be
attributed to the wide range of chemical compounds released during extraction process from seeds as well as efficiency of the applied method [30-31].

The most active compounds detected of Nigella sativa extract are the thymoquinone, dithymoquinone, thymohydroquinone, p-cymen, corvacrol, thymol and other phenolic derivatives. All extract compounds collectively observed reduce inflammation process, ontogenesis, and antitumor and antioxidant activity [32-34]. Nigella sativa was observed to induce antitumor effects in lung, breast, multiple myeloma, pancreatic, as well as gastrointestinal cancers. [35, 36] However, these active gradients were observed lethal on Serratia marcescens bacteria growth. On the other hand, other researchers observe significant increase in bacterial killing effect of Nigella sativa extract with added nonmaterial [37, 38]. Efflux pumps are transport protein in bacteria associated with elimination of toxic molecules from cell to external environment, moreover, this mechanism play essential role in reducing bacteria killing and develop bacteria resistance. The killing mechanism of Nigella Sativa extract on Serratia marcescence could be involved directly in the effect on cell membrane efflux pumps, which reduce bacteria resist and survive [39,40].

CONCLUSIONS
In conclusion: Water extraction for four hours at 60 °C is considered as potential technique for extraction active chemical ingredients of Nigella sativa seeds. The selected method in this research provided simple extraction procedure with high yield of active ingredients with reduces degradation of organic molecules result from extraction process. The selected Nigella sativa extraction method at pervious conditions was a very effective inhibitor to Serratia marcescens bacteria. From research data, Nigella Sativa extract may be help in treatment of Serratia marcescens infections. The extraction method was low cost, simple and sophisticated. However, our data provided new addition antibacterial activity to Nigella Sativa. We strongly recommended that more investigation of Nigella Sativa extract alone or mixed with other material can apply to another types of resist bacteria as well as evaluated its pharmacological action on untreated or chronic diseases [41-45].

REFERENCES
[1] Ibrahim RM, Hamdan NS, Mahmud R, Imam MU, Saini SM, Rashid SN, et al. A randomised controlled trial on hypolipidemic effects of Nigella Sativa seeds powder in menopausal women. J Transl Med. 2014;12:82. DOI: 10.1186/1479-5876-12-82
[2] Ibrahim RM, Hamdan NS, Ismail M, Saini SM, Abd Rashid SN, Abd Latiff L, et al. Protective effects of Nigella sativa on metabolic syndrome in menopausal women. Adv. Pharm Bull. 2014;4(1):29-33. DOI: 10.5681/apb.2014.005
[3] Tavakkoli A, Ahmadi A, Razavi BM, Hosseinzadeh H. Black seed (Nigella sativa) and its constituent thymoquinone as an anti-dote or a protective agent against natural or chemical toxicities. Iran J Pharm Res. 2017; 16:2-23. PMCID: PMC5963642
[4] Tavakkoli A, Mahdian V, Razavi BM, Hosseinzadeh H. Review on clinical trials of black seed (Nigella sativa) and its active constituent, thymoquinone. J Pharmacopuncture 2017; 20:107-111. DOI: 10.3831/KPI.2017.20.021.
[5] Havakhan S, Sadeghnia HR, Mosa-Al-Reza Hajzadeh NM, Roshan SS, Hosseinzadeh H, Mohareri N, et al. Effect of Nigella sativa on ischemia-reperfusion induced rat kidney damage. Iran J Basic Med Sci 2014; 17:986-992. PMCID: PMC4387234
[6] Piras A, Rosa A, Marongiu B, Porcedda S, Falconieri D, Dessi MA, et al: Chemical composition and in vitro bioactivity of volatile and fixed oils of Nigella sativa L extracted by supercritical carbon dioxide. Industrial Crops and
Antimicrobial Activity of Black Cumin Seeds

Amalia T., Bogi P., Noor H., Study of Antimicrobial Activity of Black Cumin Seeds (Nigella sativa L.) Against Salmonella typhi In Vitro, Journal of Medical & Surgical J Pathology, 2016, 1:3, 10.1016/j.jep.2015.09.022.

Investigating the Efficiency of Simple Aqueous Extract of Nigella Sativa Activity Against Serratia Marcescens

Mansi KMS. Effects of oral administration of honey based formulation of Nigella sativa seed oil in functional dyspepsia: a double blind randomized controlled clinical trial. J. Ethnopharmacol. 2015; 175: 147-52. DOI: 10.1016/j.jep.2015.09.022.

Barakat EMF, El Wakeel LM, Haggag RS. Effects of Nigella sativa on outcome of hepatitis C in Egypt. World J Gastroenterol. 2013; 19 (16): 2529-36. DOI: 10.3748/wjg.v19.i16.2529

Association between cultures of contact lens and Klebsiella pneumoniae acquisition in a neonatal intensive care unit prompting review of decontamination of laryngoscopes. J Hosp Infect 2005; 59: 68–70. DOI: 10.1016/j.jhin.2004.08.003

Antimicrobial activity of prodigiosin isolated from Serratia marcescens UFPEDA 398, World Journal of Microbiology and Biotechnology February 2015, Volume 31, Issue 2, pp 399–406. DOI: 10.1007/s11274-014-1793-y

Panel C., Arcelloni B., Comuzzi R., et.al., Quantification of gentamicin in Mueller–Hinton agar by high-performance liquid chromatography., Journal of Chromatography B: J. Biomedical Sciences and Applications., Volume 753, Issue 1, 25 March 2001, Pages 151-156. DOI: 10.1016/S0378-4347(00)00460-6

Karuppusamy S, Rajasekaran KM. High throughput antibacterial screening of plant extracts by resazurin redox with special reference to medicinal plants of Western Ghats, Glob J Pharmacol. 2009; 3 (2):63–68. ISSN 1992-0075

Mansi KMS. Effects of oral administration of water extract of Nigella sativa on serum concentrations of insulin and testosterone in alloxan-induced diabetic rats. Pak J. Biol. Sci., 2005; 8: 1152-1156.ISSN 1028-8880.
[26] Kokoska L., Havlík J., Valterova I., Sovova H., Sajftrova M., Jankovska I.: Comparison of chemical composition and antibacterial activity of Nigella sativa seed essential oils obtained by different extraction methods. J. Food Prot. 2008, 71: 2475-2480. DOI: 10.4315/0362-028X-71.12.2475.

[27] M.A. Ali, M.A. Sayeed, M.S. Alam, M.S. Yeasmin, A. M. Khan, I. I. Muhamed: Characteristics of oils and nutrient contents of Nigella sativa Linn. and Trigonella foenum-graecum seeds, Bull. Chem. Soc. Ethiop., 2012, 26, pp. 55-64. DOI: 10.4314/bcs.v26i1.6.

[28] H.J. Harzallah, E. Noumi, K. Bekir, A. Bakhrouf, T. Mahjoub: Chemical composition, antibacterial and antifungal properties of Tunisian Nigella sativa fixed oil., Afr. J. Microbiol. Res., 2012, 6, pp. 4675-4679. DOI: 10.5897/AJMR11.1073.

[29] M. M. K. Ali*, M. A. Hasan and M. R. Islam: Influence of Fertilizer Levels on the Growth and Yield of Black Cumin (Nigella sativa L.), A Scientific Journal of Krishi Foundation, The Agriculturists 13(2): 97-104(2015), DOI: 10.3329/agric.v13i2.62596.

[30] Aftab A, Asif H, Mohd, M. et. al., A review on therapeutic potential of Nigella sativa: A miracle herb., Asian Pacific Journal of Tropical Biomedicine may 2013, Volume 3, Issue 5, Pages 337-352. DOI: 10.1016/S2221-1691(13)60075-1.

[31] Selin I., Murat K., Sinem A. quantitative Analysis of Thymoquinone in nigella sativa. (Black cumin) seeds and commercial seed oils and seed oil capsules from turkey, J. Fac. Pharm. Ankara / Ankara Eciz. Fak. Derg., 2017, 41(1): 34-41.

[32] Sethi G, Ahn KS, Aggarwal BB. Targeting nuclear factor-kappa B activation pathway by thymoquinone: role in suppression of antiapoptotic gene products and enhancement of apoptosis. Mol Cancer Res. 2008;6(6):1059-70. DOI: 10.1158/1541-7786.MCR07-2088. Erratum in: Mol Cancer Res. 2018;16(9): 1441. DOI: 10.1158/1541-7786.MCR-07-2088 Published June 2008.

[33] Majeed H. M. Antimicrobial Activity of Aqueous, Ethanolic Extracts of Nigella Sativa (Black Cumin) Against Pathogenic Bacteria Isolation from Digestive Tract in Poultry, Indian journal of forensic medicine & toxicology, 2019, vol.13, issue 4, DOI:10.5958/0973-9130.2019.00360.8.

[34] Karameş, Murat; Özgür, Didem, The antibacterial and antifungal activities of commonly used herbal oils, Journal of Experimental & Clinical Medicine Apr 2020, Vol. 37 Issue 2, p47-51. 5p. http://search.ebscohost.com.

[35] Zeinab K, Mariam S, Seyede E, Arezoo S. Reduced IKK/NF-κB Expression by Nigella Sativa Extract in Breast Cancer, Middle East Journal of Cancer; April 2020; 11(2): 150-158. DOI: http://mejc.sums.ac.ir/article_46414_371c2b4210566ded0a5ce9a817ae07c.pdf.

[36] Mohammad Y. A., Investigating the protective effect of Nigella sativa against the cyclophosphamide induced genotoxicity in rats., 2020, Research Journal of Aleppo University, 138: 1-8, Feb. DOI: https://www.researchgate.net/profile/Mohammad_Abajy/publication/340128754.pdf

[37] Kamal K, Dr. Sahni Y.P., Sharma R.K., Vidhi G., Alka S. in vitro antibacterial activity of Panchgavya, Nigella sativa and Asparagus racemosus, The Pharma Innovation Journal 2019; 8(8): 307-310. DOI: http://www.thepharmajournal.com/archives/2019/vol8issue8/PartE/8-8-20-549.pdf.

[38] Ahmad A, Habeeb K, Mohd A, Arshad H, Fahd Mohammad, Khaled S. Antibacterial, Antibiofilm and Anticancer Activity of Biologically Synthesized Silver Nanoparticles Using Seed Extract of Nigella sativa, 2020, Basel, Switzerland. DOI.org/10.3390/pr8040388.

[39] Ahmad M., Ágnes T., Gabriella S., Silla M., Gabriella K., Antibacterial and Resistance Modifying Activities of Nigella sativa Essential Oil and its Active Compounds Against Listeria monocytogenes., 2018, in vivo 32: 737-743 DOI:10.21873/inivo.11302.

[40] Timea M., Ahmad M., Munira H., Annamária K, Csilla M., Gabriella K, Gabriella S., Bioactive Compounds of Nigella Sativa Essential Oils Antibacterial Agents against ChlamydiaTrachomatis D, 2019, Microorganisms 2019, 7, 370; DOI:10.3390/microorganisms7090370.

[41] Cut A., Urip H., Muhammad I., The Effect of Black Cumin Oil (Nigella Sativa Oil) Supplementation to Prevent Antiproliferative Action of Temozolomide on Hippocampal Neuronal Stem Cells., Cut Azwanidar / International Journal of Research Publications (IJRP.ORG), 2020 Volume-45, Issue-1, January 2020, DOI: 10045112020941.

[42] AysenurA., Fulya G., Lutfiye Y., Bektas U., Yasemin Celik A., Mehmet C., Mehmet N., Biogenic platinum nanoparticles using black cumin seed and their potential usage as antimicrobial and anticancer agent., Journal of Pharmaceutical and Biomedical Analysis, 2020, Volume 179, 5 February 2020, 112961., DOI.org/10.1016/j.jpba.2019.112961.
[43] Safiya B. Abdul M., A Review on Nigella sativa: A Marvel Herb, Journal of Drug Delivery and Therapeutics, 2020; 10 (2) :213-219 DOI.org/10.22270/jddt.v10i2.3913.

[44] Yamna K., Basir S., Shabana U. S., Obaid B., Aftab Am, Antiproliferative and apoptotic effects of proteins from black seeds (Nigella sativa) on human breast MCF-7 cancer cell line BMC Complementary Medicine and Therapies volume 20, Article number: 5 (2020), DOI: 10.1186/s12906-019-2804-1.

[45] Hacer A., Esra Ş, Simge A., Pınar Ş., Determination of Antimicrobial Activity of Different Essential Oils Obtained from Plants on Staphylococcus aureus Strains Isolated from Foods., 2020, vol. 8 no.4 DOI:10.24925/turjaf.v8i4.1012-1017.336

APPENDIX-A

Figure. UV –VIS scanning of Nigella sativa extracts with scanning parameters.
Table. Spectrum data report of extraction solution.

| Wavelength nm | RawData | RawData | RawData | RawData | RawData | RawData | RawData | RawData |
|---------------|---------|---------|---------|---------|---------|---------|---------|---------|
| 400           | 0.608   | 0.776   | 0.212   | 0.166   | 0.130   | 0.130   | 0.125   | 0.125   |
| 401           | 0.607   | 0.380   | 0.219   | 0.177   | 0.159   | 0.139   | 0.154   | 0.154   |
| 402           | 0.606   | 0.383   | 0.227   | 0.185   | 0.168   | 0.145   | 0.143   | 0.143   |
| 403           | 0.605   | 0.289   | 0.216   | 0.194   | 0.178   | 0.155   | 0.153   | 0.153   |
| 404           | 0.606   | 0.395   | 0.244   | 0.203   | 0.188   | 0.168   | 0.185   | 0.185   |
| 405           | 0.607   | 0.400   | 0.252   | 0.212   | 0.197   | 0.177   | 0.173   | 0.173   |
| 406           | 0.608   | 0.405   | 0.261   | 0.221   | 0.206   | 0.187   | 0.183   | 0.183   |
| 407           | 0.609   | 0.411   | 0.269   | 0.230   | 0.218   | 0.197   | 0.193   | 0.193   |
| 408           | 0.612   | 0.417   | 0.278   | 0.229   | 0.226   | 0.207   | 0.203   | 0.203   |
| 409           | 0.615   | 0.424   | 0.287   | 0.249   | 0.246   | 0.217   | 0.213   | 0.213   |
| 410           | 0.618   | 0.431   | 0.316   | 0.268   | 0.277   | 0.258   | 0.255   | 0.255   |
| 411           | 0.622   | 0.438   | 0.346   | 0.286   | 0.306   | 0.267   | 0.249   | 0.245   |
| 412           | 0.626   | 0.443   | 0.315   | 0.278   | 0.296   | 0.277   | 0.258   | 0.255   |
| 413           | 0.629   | 0.452   | 0.325   | 0.288   | 0.304   | 0.295   | 0.264   | 0.260   |
| 414           | 0.634   | 0.459   | 0.334   | 0.297   | 0.317   | 0.278   | 0.255   | 0.255   |
| 415           | 0.632   | 0.451   | 0.326   | 0.292   | 0.302   | 0.264   | 0.260   | 0.260   |
| 416           | 0.577   | 0.408   | 0.338   | 0.289   | 0.302   | 0.278   | 0.275   | 0.275   |

Copyright © 2020 Al-Mustansiriyah Journal of Science. This work is licensed under a Creative Commons Attribution-Noncommercial 4.0 International License.