

FINDING NOVEL ANTIBIOTIC SUBSTANCES FROM MEDICINAL PLANTS – ANTIMICROBIAL PROPERTIES OF *NIGELLA SATIVA* DIRECTED AGAINST MULTIDRUG-RESISTANT BACTERIA

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Received: January 16, 2017; Accepted: February 5, 2017

The progressive rise in multidrug-resistant (MDR) bacterial strains poses serious problems in the treatment of infectious diseases. While the number of newly developed antimicrobial compounds has greatly fallen, the resistance of pathogens against commonly prescribed drugs is further increasing. This rise in resistance illustrates the need for developing novel therapeutic and preventive antimicrobial options. The medicinal herb *Nigella sativa* and its derivatives constitute promising candidates. In a comprehensive literature survey (using the PubMed data base), we searched for publications on the antimicrobial effects of *N. sativa* particularly directed against MDR bacterial strains. *In vitro* studies published between 2000 and 2015 revealed that *N. sativa* exerted potent antibacterial effects against both Gram-positive and Gram-negative species including resistant strains. For instance, *N. sativa* inhibited the growth of bacteria causing significant gastrointestinal morbidity such as *Salmonella*, *Helicobacter pylori*, and *Escherichia coli*. However, *Listeria monocytogenes* and *Pseudomonas aeruginosa* displayed resistance against black cumin seed extracts. In conclusion, our literature survey revealed potent antimicrobial properties of *N. sativa* against MDR strains *in vitro* that should be further investigated in order to develop novel therapeutic perspectives for combating infectious diseases particularly caused by MDR strains.

Keywords: *Nigella sativa*, antimicrobial effects, multidrug-resistant (MDR) bacteria, medicinal plants, phytotherapy, herbal medicine

Introduction

Antimicrobial multidrug resistance

The rise in multidrug-resistant (MDR) bacteria has become a serious health problem and major challenge in developing treatment options of infectious diseases [1]. Understanding the molecular mechanisms underlying resistance development is fundamental for the attempts to overcome this issue. From a biomedical standpoint, resistance to treatment is a feature that is becoming increasingly common in bacteria, viruses, parasites, protozoa, and malignant tumor cells [2]. In the case of bacterial infections, common mechanisms leading to antibiotic resistance include the presence of drug-inactivating enzymes, modification of drug binding sites, changes to

influx and efflux mechanisms, and alterations in enzyme pathways [3].

The β -lactams constitute a group of antibiotics including penicillins, cephalosporins, monobactams, and carbapenems which inactivate glycopeptide transpeptidases, thereby inhibiting bacterial cell wall synthesis. This leads to its main bactericidal properties via cell lysis [3, 4]. This interaction is particularly useful in treating bacteria with multiple layers of peptidoglycan such as *Staphylococcus aureus* and other Gram-positive bacteria [4]. However, in recent years, there has been an increase in resistance against staphylococci in hospital settings. For instance, nosocomial methicillin-resistant *S. aureus* (MRSA) has adapted through a DNA mutation leading to alteration of the drug target site. This results in ineffective drug binding and a decrease in its bactericidal activity [5].

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Similar developments can be seen in enterococci commonly causing hospital acquired infections. The clinically relevant *Enterococcus faecium* and *faecalis* are part of the commensal microbiota lining the intestinal mucosa. Enterococci are able to spread antibiotic resistance properties through gene transfer to other susceptible bacteria. In consequence, vancomycin-resistant enterococci (VRE) have progressively become a serious problem in the clinical (particularly hospital) setting as this broad-spectrum antibiotic compound is commonly used as a reserve drug to treat intractable infections [6]. The decline in treatment options of bacterial infections has become critical in treating hospitalized patients and, therefore, calls for novel pharmacological therapeutical and preventive measures. One approach to tackle this issue is to explore the therapeutic properties of medicinal plants.

Nigella sativa – a medicinal plant

Plants have often laid the foundation of pharmaceutical drug development and allowed breakthroughs in treating diseases on a greater and more efficient scale. Notably, 60% of currently available antimicrobial and antitumoral drugs are derived from plants [7]. *N. sativa* is one of the oldest documented herbal medicinal plants and has been used for centuries in traditional Arabic medicine. Herbal treatment with this plant is already commonly applied and well known for its safety [8]. *N. sativa* seeds contain oil, protein, carbohydrate, fiber, and saponin. The fixed oil is composed of arachidonic acid, linoleic acid, oleic acid, almitoleic acid, palmitic acid, stearic acid, myristic acid, steroles, and eicosadenoic acid [9], whereas the essential oil of *N. sativa* entails nigellone, thymoquinone, thymohydroquinone, thymol, carvacrol, α - and β -pinene, d-limonene, d-citronellol, and p-cymene [10, 11].

Traditional medicine

In traditional Arabic herbal medicine, *N. sativa* has been used for a broad range of conditions including asthma, gastrointestinal dysfunctions, conjunctivitis, eczema, in-

trinsic hemorrhage, hypertension, and pyrexia [12]. In recent years, scientists have studied the therapeutic impact of *N. sativa* on cancer [13–15], hypertension [16], asthma [17], and gastrointestinal disease [18]. Some of these effects can be attributed to the extracts' ability to increase activity of intrinsic enzymes with antioxidant function such as glutathione peroxidase and catalase. The oil itself acts as a free radical scavenger and impacts the activity of cytokines, chemokines, and prostaglandins and, furthermore, modulates B cell-dependent immune pathways [19].

Given its broad health beneficial effects, we here addressed the state of the art of antimicrobial effects of *N. sativa* and its derivatives particularly against MDR bacterial strains applying a comprehensive literature survey.

Methods

Inclusion and exclusion criteria

Inclusion criteria were *in vitro* trials with bacterial strains and the effect of *N. sativa* on the inhibition of bacterial growth. *In vivo* studies and clinical trials were excluded given the small number of conducted studies so far. For the same reason, *in vitro* studies investigating the eradication properties of *N. sativa* directed against viruses and fungi were also excluded.

Search strategy

An online literature search was systematically conducted from November 15 to December 23, 2016 on the MEDLINE database PubMed in order to find relevant publications discussing the link between *N. sativa* and its effects on resistant bacterial strains and considered all publications between 2000 and 2015. By utilizing Boolean logic through the advance search history option on the PubMed database, the following steps were conducted (as summarized in Fig. 1):

Firstly, “*Nigella sativa* OR black cumin” was searched, and the Boolean operator OR ensured that synonyms were included. This method was further enhanced by employing

History		Download history Clear history	
Search	Add to builder	Query	Items found Time
#8	Add	Select 10 document(s)	10 12:56:55
#6	Add	Search (((nigella sativa OR black cumin)) AND (antimicrobial OR antibiotic)) AND antibiotic resistance	10 12:55:59
#7	Add	Search (((nigella sativa OR black cumin)) AND (antimicrobial OR antibiotic)) AND antibiotic resistance Filters: published in the last 10 years	8 12:55:53
#5	Add	Search antibiotic resistance	167180 12:54:49
#4	Add	Search ((nigella sativa OR black cumin)) AND (antimicrobial OR antibiotic)	155 12:53:47
#3	Add	Search antimicrobial OR antibiotic	1583788 12:53:15
#2	Add	Search nigella sativa OR black cumin	964 12:52:55
#1	Add	Search nigella sativa	881 12:52:42

Fig. 1. Boolean logic search results on PubMed

the MeSH (Medical Subject Headings) term, and therefore, other variations of the herbs' name such as black caraway and *kalonji* could be found in the database. Secondly, the following search term "antimicrobial OR antibiotic" was included in order to find studies focussing on antibiotic properties. Thirdly, the term "antibiotic resistance" was used to ensure that investigations on resistant bacteria were included. Finally, to narrow down the search, all three search terms were united through the Boolean operator "AND" in following manner: "(*Nigella sativa* OR black cumin) AND (antimicrobial OR antibiotic) AND (antibiotic resistance)".

In consequence, ten items were systematically found. By evaluating all ten articles according to the inclusion requirements, three were rejected since one article described a clinical trial, another study used *Cuminum cyminum* instead of the black seed herb, and the full article of an *in vitro* study was not available. Hence, a total of seven articles were included finally.

Data extraction

By using Microsoft Excel, information gained from respective articles was extracted according to the following criteria: type of extract used, scientific method, targeted bacteria (Gram-positive and Gram-negative), control, main findings, and references.

Results

Antibiotic properties of *N. sativa*

N. sativa oil and crude extract were analyzed for their effects on the growth of six *Salmonella* variants including *S. paratyphi*, *S. enteritidis*, *S. typhimurium*, *S. heidelberg*, and *S. agona* applying the well diffusion assay and broth dilution method [20]. By utilizing antibiotic susceptibility tests, all tested *Salmonella* strains were exposed to the synthetic antibiotic compounds ceftriaxone and ciprofloxacin and proven to be resistant. Remarkably, all resistant *Salmonella* strains were susceptible to *N. sativa* crude extract, while for the oil extract, this was the case in 90% of the strains. All of the against black seed oil tested *Salmonella* strains displayed distinct average inhibition zones, namely, 19 mm against *S. paratyphi*, 17 mm against *S. enteritidis*, 30 mm against *S. typhimurium*, 15 mm against *S. heidelberg*, and 23 mm against *S. agona* [20].

In another *in vitro* study, aqueous extracts of the black cumin seeds were used against seven strains of the Gram-negative pathogen *Helicobacter pylori* known to cause gastric and duodenal ulcers as well as gastroduodenal adenocarcinomas in 5–10% of infected individuals. Six of the strains had been isolated from hospitalized patients suffering from peptic ulcers [21]. The aqueous extract was produced by boiling *N. sativa* seeds in sterile distilled water (100 mg/ml) and filtered through sterile filter paper.

Standard bacterial killing curves revealed that bacterial growth was completely inhibited in all *H. pylori* strains after 60 minutes incubation time [21].

In another set of experiments, the antibacterial activities of black cumin essential oil (EO) were tested against three Gram-positive and three Gram-negative bacterial strains, namely, *S. aureus*, *Bacillus cereus*, *Listeria monocytogenes*, and *S. typhimurium*, *Escherichia coli*, *Pseudomonas aeruginosa*, respectively [11]. The synthetic antibiotic ceftriaxone and a naturally derived antimicrobial named eugenol served as control compounds. For antibacterial survey, the agar disk diffusion method was applied with concentrations of 100, 200, 300, 400, and 500 µg compound per well. The EO microemulsion displayed its highest antibacterial activity in a minimum concentration of 100 µg EO per well against *S. aureus* and *B. cereus* with inhibition zones of 15.7 mm and 14.0 mm, respectively. As compared to eugenol microemulsion, black cumin microemulsion exerted higher antibiotic activity against *S. aureus*, *B. cereus*, and *S. typhimurium* in all concentrations [11].

No significant differences, however, could be observed in growth inhibitions against *S. typhimurium* at 300 µg/well exerted by *N. sativa* EO (18.0 mm inhibition zone) as compared to ceftriaxone (19.3 mm). Interestingly, at concentrations of 400 µg and 500 µg, the antibacterial efficacy of EO (26.5 mm) against respective bacterial strains was shown to be even more potent than ceftriaxone (23.7 mm). At 500 µg/well, *N. sativa* EO was found effective against *E. coli* as indicated by a mean inhibition zone of 30.3 mm, that was comparable to that exerted by ceftriaxone (31.3 mm) at the same concentration. *L. monocytogenes* and *P. aeruginosa*, however, were tested resistant to EO at all applied concentrations [11].

Another study using the agar diffusion method determined the growth of inhibition against *S. aureus*, two *B. cereus* strains, *E. coli* and *P. aeruginosa* by both *N. sativa* EO and crude oil extract applied in 500 mg/ml concentration [22]. The synthetic antibiotic gentamycin was used as negative control. The petroleum ether extract (500 mg/ml) exceeded the inhibition zone exerted by gentamycin in two tested *B. cereus* strains, namely, 40.0 ± 2.3 mm and 44.0 ± 0.3 mm against *B. cereus* strain ATCC 10987 and a *B. cereus* laboratory strain, respectively, following petroleum ether extract as compared to 30.0 ± 1.2 mm (against *B. cereus* ATCC 10987) and 25.0 ± 1.0 mm (against laboratory derived *B. cereus* strain) following gentamycin application. Both *P. aeruginosa* and *S. aureus*, however, were resistant to both plant extracts. Hence, the crude oil extract displayed higher antimicrobial activities as compared to the EO extract [22].

In a further study, the effect of black cumin seed oil was investigated in several concentrations on MDR *S. aureus* strains that had been isolated from diabetic wounds employing the well diffusion method [23]. Eight *S. aureus* isolates out of 19 strains tested were susceptible to *N. sativa* oil in its undiluted form, whereas another four isolates displayed susceptibility at concentrations ranging from

Table 1. Antibacterial effects of *Nigella sativa*

Extract	Method	Gram-positive bacteria	Gram-negative bacteria	Control	Main findings	References
Aqueous extract of seeds	Standard killing curve	Not available	<i>H. pylori</i>	<i>H. pylori</i> with sterile distilled water	100% of inhibition of growth after 60-min incubation against all seven strains.	[21]
Oil and crude extract	Well diffusion assay and broth dilution method	Not available	Six strains of <i>Salmonella</i>	Antibiotic susceptibility test against 50 and 100 µg/ml ciprofloxacin and ceftriaxone	All <i>Salmonella</i> strains were susceptible to the crude extract. 90% of resistant <i>Salmonella</i> strains were sensitive to the oil extract.	[20]
Essential oil extract in microdilution	Agar well diffusion	<i>S. aureus</i> , <i>B. cereus</i> , <i>L. monocytogenes</i>	<i>S. typhimurium</i> , <i>E. coli</i> , <i>P. aeruginosa</i>	Eugenol microemulsion and ceftriaxone	EO showed its high antibacterial activity in the concentration of 100 µg EO/well against <i>S. aureus</i> and <i>B. cereus</i> . At 500 µg EO/well effective against <i>E. coli</i> (20.2 mm) and <i>S. typhimurium</i> (26.5 mm). <i>L. monocytogenes</i> and <i>P. aeruginosa</i> were resistant to EO at all concentrations.	[11]
Essential oil and petroleum ether extract	Agar well diffusion	<i>S. aureus</i> , two strains of <i>B. cereus</i>	<i>E. coli</i> and <i>P. aeruginosa</i>	Gentamycin sulfate	The petroleum ether extract (500 mg/ml) exceeded the zone of inhibition of gentamycin in both tested strains of <i>B. cereus</i> . <i>P. aeruginosa</i> was resistant to both plant extracts.	[22]
Oil extract	Disc diffusion and well diffusion method	MRSA	Not available	Antibiotic susceptibility test	62% of tested MRSA strains were susceptible to <i>N. sativa</i> oil.	[23]
Ethanollic extract	Broth microdilution test and agar well diffusion test	VRSA	Not available	Linezolid, moxifloxacin, clindamycin	Highest inhibition of growth (26 mm) was achieved at 20 mg/well (ethanollic extract) for five VRSA isolates.	[24]
Ethanollic extract	Disc and agar dilution method	MRSA	Not available	Unknown	All MRSA strains were susceptible to the ethanollic extract at 4 mg/disc.	[25]

200 mg/ml to 800 mg/ml. The remaining isolates, that is, eleven in total, exhibited resistance to the oil in all dilutions. Thus, 62% of the tested isolates were sensitive to *N. sativa* oil [23].

Results from another *in vitro* experiment revealed a mean minimum bactericidal concentration of 819.2 µg/ml of *N. sativa* ethanolic extract against vancomycin-resistant *S. aureus* (VRSA). The five isolates were taken from clinical samples, and the agar well diffusion method was employed. At 20 mg/well of the ethanolic extract, the average inhibition zone for all five VRSA isolates was 22.2 mm [24].

Furthermore, antibacterial activities of black caraway ethanol extract against MRSA strains were surveyed. Following exposure to a concentration of 4 mg in the agar diffusion assay, all MRSA strains were shown to be susceptible to the extract with a minimum inhibitory concentration ranging from 0.2 to 0.5 mg/ml [25]. The above-mentioned findings are summarized in *Table 1*.

Antibacterial constituents of N. sativa

The chemical constituents of *N. sativa* essential oil gained by hydrodistillation were analyzed by gas chromatography [11]. The antimicrobial properties of black cumin seed oil were shown to depend on the concentration of the active compounds thymoquinone and thymohydroquinone. Thymoquinone made up 52.6% of the EO extract, thus, constituting the main active component, whereas p-cymene, the second dominating compound, was found to make up to 25% of the EO [11].

Notably, thymoquinone displays a critical antibiotic role as it is able to decrease bacterial resistance to distinct antimicrobial compounds. One common mechanism of resistance is an increased antibiotic clearance from the cell via efflux pumps, thereby decreasing the intracellular concentration of the respective antimicrobial compound. Thymoquinone was described to inhibit the activity of bacterial efflux pumps and consequently to interrupt an important process leading to antibiotic resistance [11]. Besides, thymoquinone exerted potent antibiofilm directed properties against both Gram-positive and Gram-negative pathogens [11].

Discussion

Main findings of the literature survey

Our comprehensive literature survey revealed that the medicinal herb *N. sativa* exhibited antimicrobial properties against bacterial strains that were shown to be resistant against commonly used synthetic antibiotic compounds. Both Gram-positive and Gram-negative bacteria were susceptible to *N. sativa* at different concentrations. Enteropathogens such as *Salmonella*, *H. pylori*, and *E. coli* strains were inhibited in their growth. The same applies

to *B. cereus*, *S. aureus*, and, strikingly, to MDR *S. aureus* strains including MRSA and even VRSA. However, *L. monocytogenes* and *P. aeruginosa* were shown to exert resistances directed against black cumin seed extracts. Such resistances against *N. sativa* may arise given the distinct adaptability and metabolic versatility inherent in certain bacterial strains [11]. The positive effects in the conducted *in vitro* studies, however, strongly support the application of this medicinal plant as a potential drug for combating bacterial infectious diseases.

Limitations of the literature survey

Conclusions should be rather carefully drawn from the afore-discussed papers given the lack of consistencies in variables such as drug concentrations and other confounding constituents. This limitation is due to the natural variation in the concentrations of compounds found in plant-derived extracts. Furthermore, the relatively small number of papers exhibit another limitation.

Future perspectives and in vivo applications

The antimicrobial activities exerted by thymoquinone may be potentiated by synergistically acting agents present in the oil such as p-cymene, which needs to be further explored. Fully understanding the mechanisms underlying the antimicrobial effects of thymoquinone and other components that might be relevant to the distinct antimicrobial impact will help to formulate effective synthetic antibiotic compounds, which will provide novel therapeutic options of combating bacterial infections caused even by MDR strains.

In order to achieve this, further *in vitro* studies applying comparable extracts under standardized methods are required for allowing more conclusive statements regarding the potent antimicrobial effects of *N. sativa* and its derivatives in pharmacological formulations.

Important steps towards potential pharmacological application were elucidating antimicrobial efficacies of *N. sativa* in *in vivo* studies. In this context, one previous study addressed the impact of *N. sativa* seed on intestinal *E. coli* colonization and jejunal morphology in laying hens [26]. The *in vivo* data revealed that best results for intestinal health were achieved following application of 2% *N. sativa*, resulting in a significant reduction in ileal *E. coli* loads [26]. Moreover, thymoquinone sufficiently preserved intestinal epithelial barrier function and prevented from bacterial translocation. These effects were supported by another *in vivo* study including rats with intestinal obstruction, a condition associated with motility dysfunction, intestinal bacterial overgrowth, and mucosal disruption [27]. Upon intraperitoneal treatment with 10 mg thymoquinone per kg body weight, rats displayed a decrease in oxidative stress and inflammatory cytokines and were prevented from inflammatory damage to both liver and intestine [27].

Furthermore, in a clinical trial, patients suffering from non-ulcer dyspepsia were subjected to *N. sativa* in order to treat underlying *H. pylori* infection and compared to a cohort receiving a well-established triple therapy with clarithromycin, amoxicillin, and omeprazole. The patients were randomly included into the following groups: 1) triple therapy; 2) 1 g *N. sativa* plus 40 mg omeprazole; 3) 2 g *N. sativa* plus 40 mg omeprazole; 4) 3 g *N. sativa* plus 40 mg omeprazole, for 4 weeks, respectively. Remarkably, the combination of 2 g *N. sativa* plus 40 mg omeprazole was shown to be most effective in eradicating *H. pylori* [28]. Future clinical research should further expand these promising findings to diseases caused by other microbial pathogens.

In conclusion, our literature survey revealed potent antimicrobial properties of *N. sativa* against MDR bacteria *in vitro* providing promising perspectives for the development of novel therapeutic options combating infections that are particularly caused by MDR bacterial strains.

Funding sources

There has been no source of funding.

Conflict of interest

Stefan Bereswill and Markus M. Heimesaat are Editorial Board members.

References

- Fernández J, Bert F, Nicolas-Chanoine MH: The challenges of multi-drug-resistance in hepatology. *J Hepatol* 65, 1043–1054 (2016)
- Avner BS, Fialho AM, Chakrabarty AM: Overcoming drug resistance in multi-drug resistant cancers and microorganisms: a conceptual framework. *Bioengineered* 3, 262–270 (2012)
- Xia J, Gao J, Tang W: Nosocomial infection and its molecular mechanisms of antibiotic resistance. *Biosci Trends* 10, 14–21 (2016)
- Bush K, Bradford PA: β -Lactams and β -lactamase inhibitors: an overview. *Cold Spring Harb Perspect Med* 6(8), pii: a025247 (2016)
- Joo HS, Otto M: Mechanisms of resistance to antimicrobial peptides in staphylococci. *Biochim Biophys Acta* 1848, 3055–3061 (2015)
- Reyes K, Bardossy AC, Zervos M: Vancomycin-resistant enterococci: epidemiology, infection prevention, and control. *Infect Dis Clin North Am* 30, 953–965 (2016)
- Calixto JB: Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). *Braz J Med Biol Res* 33, 179–189 (2000)
- Burits M, Bucar F: Antioxidant activity of *Nigella sativa* essential oil. *Phytother Res* 14, 323–328 (2000)
- El-Tahir KE-DH, Bakeet DM: The black seed *Nigella sativa* Linnaeus 21us-A mine for multi cures: a plea for urgent clinical evaluation of its volatile oil. *J Taibah Univ Med Sci* 1, 1–19 (2006)
- Nickavar B, Mojab F, Javidnia K, Amoli MA: Chemical composition of the fixed and volatile oils of *Nigella sativa* L. from Iran. *Z Naturforsch C* 58(9–10), 629–631 (2003)
- Shaaban HA, Sadek Z, Edris AE, Saad-Hussein A: Analysis and antibacterial activity of *Nigella sativa* essential oil formulated in microemulsion system. *J Oleo Sci* 64, 223–232 (2015)
- Ali BH, Blunden G: Pharmacological and toxicological properties of *Nigella sativa*. *Phytother Res* 17, 299–305 (2003)
- Salim EI, Fukushima S: Chemopreventive potential of volatile oil from black cumin (*Nigella sativa* L.) seeds against rat colon carcinogenesis. *Nutr Cancer* 45, 195–202 (2003)
- Singh AP, Moniaux N, Chauhan SC, Meza JL, Batra SK: Inhibition of MUC4 expression suppresses pancreatic tumor cell growth and metastasis. *Cancer Res* 64, 622–630 (2004)
- Banerjee S, Kaseb AO, Wang Z, Kong DM, Mussop P, Subhash S, Fazlul HM, Ramzi M: Antitumor activity of gemcitabine and oxaliplatin is augmented by thymoquinone in pancreatic cancer. *Cancer Res* 69, 5575–5583 (2009)
- Dehkordi FR, Kamkhah AF: Antihypertensive effect of *Nigella sativa* seed extract in patients with mild hypertension. *Fundam Clin Pharmacol* 22, 447–452 (2008)
- Boskabady M, Mohsenpoor N, Takaloo L: Antiasthmatic effect of *Nigella sativa* in airways of asthmatic patients. *Phytomedicine* 17, 707–713 (2010)
- El-Abhar H, Abdallah D, Saleh S: Gastroprotective activity of *Nigella sativa* oil and its constituent, thymoquinone, against gastric mucosal injury induced by ischaemia/reperfusion in rats. *J Ethnopharmacol* 84, 251–258 (2003)
- Gholamnezhad Z, Keyhanmanesh R, Boskabady MH: Anti-inflammatory, antioxidant, and immunomodulatory aspects of *Nigella sativa* for its preventive and bronchodilatory effects on obstructive respiratory diseases: a review of basic and clinical evidence. *J Funct Food* 17, 910–927 (2015)
- Sarwar A, Latif Z: GC-MS characterisation and antibacterial activity evaluation of *Nigella sativa* oil against diverse strains of *Salmonella*. *Nat Prod Res* 29, 447–451 (2015)
- O'Mahony R, Al-Khtheeri H, Weerasekera D, Fernando N, Vaira D, Holton J, Bassett C: Bactericidal and anti-adhesive properties of culinary and medicinal plants against *Helicobacter pylori*. *World J Gastroenterol* 11, 7499–7507 (2005)
- Bacha K, Tariku Y, Gebreyesus F, Zerihun S, Mohammed A, Weiland-Bräuer N, Schmitz RA, Mulat M: Antimicrobial and anti-Quorum Sensing activities of selected medicinal plants of Ethiopia: implication for development of potent antimicrobial agents. *BMC Microbiol* 16, 139 (2016)
- Emeka LB, Emeka PM, Khan TM: Antimicrobial activity of *Nigella sativa* L. seed oil against multi-drug resistant *Staphylococcus aureus* isolated from diabetic wounds. *Pak J Pharm Sci* 28, 1985–1990 (2015)
- Liaqat F, Sheikh AA, Nazir J, Hussain T, Rabbani M, Shaheen AY, Muhammed J: Report-isolation identification and control of vancomycin resistant *Staphylococcus aureus*. *Pak J Pharm Sci* 28, 997–1004 (2015)
- Hannan A, Saleem S, Chaudhary S, Barkaat M, Arshad MU: Anti-bacterial activity of *Nigella sativa* against clinical isolates of methicillin resistant *Staphylococcus aureus*. *J Ayub Med Coll Abbottabad* 20, 72–74 (2008)

26. Boka J, Mahdavi A, Samie A, Jahanian R: Effect of different levels of black cumin (*Nigella sativa* L.) on performance, intestinal *Escherichia coli* colonization and jejunal morphology in laying hens. *J Anim Physiol Anim Nutr* 98, 373–383 (2014)
27. Kapan M, Tekin R, Onder A, Firat U, Evliyaoglu O, Taskesen F, Arikanoğlu Z: Thymoquinone ameliorates bacterial translocation and inflammatory response in rats with intestinal obstruction. *Int J Surg* 10, 484–488 (2012)
28. Salem EM, Yar T, Bamosa A, Al-Quorain A, Yasawy MI, Alsulaiman RM, Randhawa MA: Comparative study of *Nigella Sativa* and triple therapy in eradication of *Helicobacter Pylori* in patients with non-ulcer dyspepsia. *Saudi J Gastroenterol* 16, 207–214 (2010)