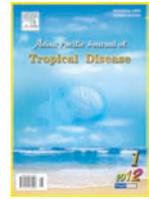


Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd



Document heading

Antibacterial activity of honey alone and in combination with *Nigella sativa* seeds against *Pseudomonas aeruginosa* infection

Meslem Abdelmalek¹, Ahmed Moussa^{1*}, Djebli Noureddine², Aissat Saad¹

¹ Institute of Veterinary Sciences University, Ibn–khaldoun Tiaret (14000), Algeria

² Departments of Biology, Faculty of Sciences, Mostaganem University, Algeria

ARTICLE INFO

Article history:

Received 15 June 2012

Received in revised form 27 June 2012

Accepted 18 October 2012

Available online 28 October 2012

Keywords:

Honey

Nigella sativa

Antibacterial activity

Pseudomonas aeruginosa

ABSTRACT

Objective: To evaluate the *in vitro* activities, of three honeys sample, and *Nigella sativa* (*N. sativa*) against *Pseudomonas aeruginosa* (*P. aeruginosa*) alone and in combination. **Methods:** The antibacterial test and minimum inhibition concentration (MIC) was determined by using agar well diffusion and dilution methods respectively against *P. aeruginosa*. **Results:** The MIC for the three varieties of honey without *N. sativa* against *P. aeruginosa* ranged between 46% and 50% (v/v). Addition of *N. sativa* (8%) resulted in synergistic bactericidal activity. An MIC drop was noticed with each variety and it ranged between 77.77% and 84.21%. **Conclusions:** These antibacterial properties would warrant further studies on the clinical applications of *N. sativa* and honey against *P. aeruginosa*.

1. Introduction

Infectious diseases still represent an important cause of morbidity and mortality among humans, especially in developing countries[1]. In recent years, human pathogenic microorganisms have developed resistance in response to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases[2]. Therefore, alternative antimicrobial strategies are urgently needed, and thus this situation has led to a re-evaluation of the therapeutic use of ancient remedies, such as plants and plant-based products, including honey and *Nigella sativa*[3]. The application of honey in medicine has recently been rediscovered and is gaining acceptance as an antibacterial agent for the treatment of ulcers, wounds, and other surface infections. Honey has also been shown to be effective in rapidly responding to standard antiseptic and antibiotic therapy and as a method of accelerating wound healing[4]. Honey is such a complex and variable natural product that the search for specific inhibitors has been

extensive[5]. The antibacterial activity of honey has been confirmed by numerous scientific studies[6–8]. Antibacterial activity has been demonstrated against both Gram-positive and Gram-negative, both aerobic and anaerobic types.

Nigella sativa L. (*N. sativa*) also known as black cumin is an annual herbaceous plant belonging to the Ranunculaceae family[9]. The plant is indigenous to Mediterranean areas, though it is grown in other parts of the world as well[10]. Its seeds have played an important role over the years in ancient Islamic system of herbal medicine and in Algeria where they have been traditionally used in folk medicine. Traditionally, it is used as a natural remedy for a number of illnesses that include asthma, cough, hypertension, bronchitis, diabetes, headache, eczema, fever, inflammations, and other diseases[11]. Different crude extracts of *N. sativa* have shown effectiveness against multiantibiotic resistance bacterial isolates[12]. The antimicrobial effects of *N. sativa* seeds against different pathogenic microbes were investigated. The importance of *N. sativa* and honey cannot be over emphasized as regards their rule in health remedy. Therefore this study detailed the antibacterial activities of honey and *N. sativa* on selected pathogenic bacteria.

2. Material and methods

2.1. Honey samples

*Corresponding author: Ahmed Moussa, Institute of Veterinary Sciences University Ibn–Khalidoun Tiaret (14000), Algeria.

Tel: +213 65234059

Fax: +213 46425001

E-mail: moussa7014@yahoo.fr

Foundation Project: This work was financially supported by project CNEPRU, Institute of Veterinary Sciences (IVS), University Ibn–Khalidoun (TIARET), Algeria[Grant No.F023 2009 / 0009].

During the 2009 flowering seasons, three honey samples were gathered and provided by various bee-keepers from two area different from the Algeria west. These honey samples were aseptically collected in sterile screwed cups and kept in a cool and dry place (at room temperature) overnight before they were finally transported to the laboratory.

2.2. Plant materials and preparation

N. sativa seeds were obtained from the local seed supplier. The seeds were crushed manually in a mortar with a pestle. A volume of 100 mL of distilled water was added to 20 g of dry powder. It was vortexed continuously until there was no further change in color of the solution. This solution was centrifuged at 800 g for 15 min. The supernatant (brownish-orange in colour) was filtered through Whatman filter No. 4 and stored at 4 °C in sterile tubes until use.

2.3. Test organism

Micro-organism was obtained from the Department of Biomedicine, Institute of Veterinary Science University Ibn-Khaldun, Algeria.

2.4. Preparation of bacterial inoculum

Stock cultures were maintained at 4 °C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loop full of cells from each stock culture of *Pseudomonas aeruginosa* (*P. aeruginosa*) to test tubes of nutrient agar medium and incubating without agitation for 24 h at 37 °C. The cultures were diluted with fresh nutrient agar broth to achieve optical densities corresponding to 2.0×10^6 colony forming units (CFU/mL)[13].

2.5. Antibacterial assay

Table 1
Combined activity of *N. sativa* and the three varieties of honey against *P. aeruginosa*.

Honeysample	Honey only % (vol/vol)		MIC of the mixture of honey and <i>N. sativa</i> % (vol/vol) (H:NS)					[Honey:NS] DZ (mm)	Honey MIC drop %
	MIC	DZ (mm)	1%	2%	4%	6%	8%		
H1	19	4	19	19	19	19	3:12	>4 mm	84.21%
H2	10	6	10	10	10	10	2:14	>6 mm	80.00%
H3	9	5	9	9	9	9	2:10	>6 mm	77.77%
Control (Water)	0	0	0	0	0	0	0:0	0 mm	0.00%

NS: *N. sativa*.

4. Discussion

P. aeruginosa is a major nosocomial pathogen, particularly dangerous to cystic fibrosis patients and populations having weak immune system. In wounds, *P. aeruginosa* has emerged as a multidrug-resistant organism that gives rise to persistent infections in burns patients and chronic venous leg ulcers[14–16]. Novel antimicrobial interventions are needed. Natural medicinal products have been used for millennia to treat multiple ailments. Although many have been superseded by conventional pharmaceutical approaches, there is currently resurgence of interest by physicians in natural products. *N. sativa* seeds play an

Antibacterial activity was measured using a well diffusion method according to the National Committee for Clinical Laboratory Standard (NCCLS 1999). Briefly, Petri plates containing 20 mL of nutritive agar medium were inoculated with a 24 h culture of the bacterial strains. Wells (8 mm diameter) were punched in the agar and filled with 30 μ L of *N. sativa* or honey. The second step of a mixture of 30 μ L *N. sativa* and honey has been introduced into the wells. Triplicates of each plate have been done. The plates were incubated at 37 °C for 24 h. The antibacterial activity was assessed by measuring the diameter of the area in which bacterial growth was inhibited around the well. The average of three replicates for honey, *N. sativa*, and combination were calculated.

The controls were set up with equivalent quantities of water.

2.5.1. Minimum inhibitory concentration measurement of *N. sativa* and honey samples

Increased concentrations of honey (1–50% vol/vol) and *N. sativa* from 2%, 4%, 6% and 8% were incorporated into nutritive agar media to test their efficiency against *P. aeruginosa*. Each plate with final volume of honey and media of 5 mL was inoculated and incubated at 37 °C for 24 h. The MIC was determined by finding the plates with the lowest concentration of honey on which the strain didn't grow. All MIC values were expressed in % (v/v).

3. Results

The additive effect between honey and *N. sativa* as regards our three varieties of honeys studied against *P. aeruginosa* showed that the MIC for the three varieties of honeys were by decreasing order of effect; 3% (v/v) and 12% (w/v), 2% (v/v) and 14% (w/v), and 2% (v/v) and 10% (w/v).

important role in folk medicine and some of its major constituents are reported to be pharmacologically active[17]. Phytochemical studies of the seeds have revealed the presence of volatile oil (1.5%), fixed oil (37.5%), nigellin, melanthin, arabic acid, carvene, carvone, cymene[18], thymohydroquinone and thymoquinone[19]. It has been reported that crude extracts and essential oil possess antibacterial activity against several bacteria[20–22].

Several bioactive compounds have been identified in honey which contributed to its antibacterial action. The commonly accepted list of contributors include osmolarity[23,24], hydrogen peroxide[25], polyphenols[26], antioxidants[27] antibiotic peptides[28], and recently, Maillard reaction products[27].

Honey and *N. sativa* has been found to possess antibacterial activity and this has been attributed to specific chemicals in the honey and *N. sativa*[3]. The results of this study show that adding honey to *N. sativa* increases the antibacterial effect against *P. aeruginosa* (Table 1).

Its combination with *N. sativa* displayed valued potency on the test organisms than when used in single form. This emphasised that combination of two or more substances with medicinal values could be better if their components will not cause a reaction that could cause health disaster than healing. The exact mechanism of combination between medicinal plants and honey requires further investigation. It is therefore concluded that honey and *N. sativa* combinations due to their synergistic effect have potential to be used in the treatment of *P. aeruginosa* infections.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

Authors thank Staff of Tiaret University for providing material.

References

- Ghaleb A, Yousef S, Kamel A. Effect of ethanolic extract of *Ecballium elaterium* against *Staphylococcus aureus* and *Candida albicans*. *Asian Pac J Trop Biomed* 2011; **1**: 456–460.
- Elumalai EK, Ramachandran M, Thirumalai T, Vinothkumar P. Antibacterial activity of various leaf extracts of *Merremia emarginata*. *Asian Pac J Trop Biomed* 2011; **1**: 406–408.
- Hasan A. A, Balkees A Bakhotmah, Laïd B. *In vitro* susceptibility of diabetic wound bacteria to mixtures of honey, *Commiphora molmol* and *Nigella sativa*. *The Open Nutraceuticals Journal* 2011; **4**: 172–175.
- Adewumi AA, Ogunjinmi AA. The healing potential of honey and propolis lotion on septic wounds. *Asian Pac J Trop Biomed* 2011; **1**: S55–S57.
- Manisha Deb M, Shyamapada M. Honey: its medicinal property and antibacterial activity. *Asian Pac J Trop Biomed* 2011; **1**: 154–160.
- Mandal S, Deb Mandal M, Pal NK, Saha K. Antibacterial activity of honey against clinical isolates of *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella enterica* serovar Typhi. *Asian Pac J Trop Med* 2010; **3**: 961–964.
- Sherlock O, Dolan A, Athman R, Power A, Gethin G, Cowman S, et al. Comparison of the antimicrobial activity of ulmo honey from Chile and manuka honey against methicillin-resistant *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. *BMC Complement Alternat Med* 2010; **10**: 47.
- Hanene JH, Bochra K, Guido F, Amina B, Touhami M. Chemical composition, antimicrobial potential against cariogenic bacteria and cytotoxic activity of Tunisian *Nigella sativa* essential oil and thymoquinone. *Food Chem* 2011; **129**: 1469–1474.
- Ali K, Hasanah MG, Ali Y, Yogeshini R, Ali G. Physicochemical characteristics of *Nigella* seed (*Nigella sativa* L.) oil as affected by different extraction methods. *J Am Oil Chem Soc* 2011; **88**: 533–540.
- Ali BH, Blunden G. Pharmacological and toxicological properties of *Nigella sativa*. *Phytother Res* 2003; **17**: 299–305.
- Morsi NM. Antimicrobial effect of crude extracts of *Nigella sativa* on multiple antibiotics-resistant bacteria. *Acta Microbiol Pol* 2000; **49**(1): 63–74.
- Bissa S, Avinash B, Bohra A. Antibacterial potential of three naked-seeded (Gymnosperum) plants. *Nat Prod Rad* 2008; **7**(5): 420–425.
- National Committee for Clinical Laboratory Standards (NCCLS). *Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals*. Pennsylvania, USA: NCCLS; 1999.
- Branski LK, Al-Mousawi A, Rivero H, Jeschke MG, Sanford AP, Herndon DN. Emerging infections in burns. *Surg Infect* 2009; **10**(5): 389–397.
- Keen EF 3rd, Robinson BJ, Hospenthal DR, Aldous WK, Wolf SE, Chung KK, et al. Prevalence of multidrug-resistant organisms recovered at a military burn center. *Burns* 2010; **36**(6): 819–825.
- Fazli M, Bjarnsholt T, Kirketerp-Møller K, Jørgensen B, Andersen AS, Kroghfelt KA, Givskov M, Tolker-Nielsen T. Nonrandom distribution of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in chronic wounds. *J Clin Microbiol* 2009; **47**(12): 4084–4089.
- Suresh Kumar TV, Negi PS, and Udaya Sankar K. Antibacterial Activity of *Nigella sativa* L. Seed Extracts. *Br J Pharmacol Toxicol* 2010; **1**(2): 96–100.
- Nadkarni K. *Crocus sativus*, *Nigella sativa*. In: Nadkarni KM, editor. *Indian materia medica*. India: Popular Prakashan; 1976, p. 386–411.
- Houghton PJ, Zarka R, de las Heras B, Hoult JR. Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. *Planta Medica* 1995; **61**(1): 33–36.
- Ali NA, Julich WD, Kusnick C, Lindequist U. Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. *J Ethnopharmacol* 2001; **74**(2): 173–179.
- El-Kamali HH, Ahmed AH, Mohammed AAM. Antibacterial properties of essential oils from *Nigella sativa* seeds, *Cymbopogon citratus* leaves and *Pulicaria undulata* aerial parts. *Fitoterapia* 1998; **69**: 77–78.
- Mouhajir F, Pedersen JA, Rejdali M, Towers GHN. Antimicrobial thymohydroquinones of Moroccan *Nigella sativa* seeds detected by electron spin resonance. *Pharm Biol* 1999; **37**, 391–395.
- Wahdan HAL. *Causes of the antimicrobial activity of honey*. *Infection* 1998; **26**: 26–31.
- Bose B. Honey or sugar in treatment of infected wounds? *Lancet* 1982; **1**(8278): 963.
- Brudzynski K, Abubaker K, Miotto D. Unraveling a mechanism of honey antibacterial action: Polyphenol/H₂O₂-induced oxidative effect on bacterial cell growth and on DNA degradation. *Food Chem* 2012; **133**: 329–336.
- Aljadi AM, Yusoff KM. Isolation and identification of phenolic acids in Malaysian honey with antibacterial properties. *Turk J Med Sci* 2003; **3**: 229–236.
- Brudzynski K, Miotto D. Honey melanoidins. Analysis of a composition of the high molecular weight melanoidin fractions exhibiting radical scavenging capacity. *Food Chem* 2011; **127**: 1023–1030.
- Kwakman PHS, de Boer L, Ruyter-Spira CP, Creemers-Molenaar T, Helsen JPF, Vandenbroucke-Grauls CMJE, et al. Medical-grade honey enriched with antimicrobial peptide has enhanced activity against antibiotic-resistant pathogens. *Eur J Clin Microbiol Infect Dis* 2011; **30**: 251–257.