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THE ANTIBACTERIAL ACTIVITY OF *NIGELLA SATIVA* AGAINST MULTI-DRUG RESISTANT *PSEUDOMONAS AERUGINOSA* ISOLATED FROM DIABETIC WOUND INFECTIONS

Wagih A. El-Shouny
Botany Department, Faculty of Science, Tanta University, 31527 Tanta, Egypt

Sameh S. Ali
Botany Department, Faculty of Science, Tanta University, 31527 Tanta, Egypt
Biofuels Institute, School of the Environment and Safety Engineering at Jiangsu University, Zhenjiang, 212013, China
samh_samir@science.tanta.edu.eg

Shymaa F. Fayed
Botany Department, Faculty of Science, Tanta University, 31527 Tanta, Egypt

Abstract

Thirty-five specimens were isolated from diabetic patients with superficial and deep wounds. The isolates of Pseudomonas aeruginosa were selected from cetrimide agar plates supplemented with nalidixic acid. P. aeruginosa were subjected to in vitro evaluation of antibiotic sensitivity test using antibiotics from different classes. Multi-drug resistant P. aeruginosa (MDRPA) were

selected for further tests and multiple antibiotic resistance (MAR) index *was calculated. Eleven commercial essential oils (EOs) were chosen to evaluate their activities as antimicrobial agents against MDRPA. The sensitivity was determined using agar disc diffusion method. The black seed oil (Nigella sativa) showed a wide spectrum of inhibition against MDRPA3. The characterization of Nigella sativa was conducted by GC-MS and FT-IR which showed the antibacterial activity and safety of this oil.*

Keywords
Antibacterial Activity, Essential Oil, Multidrug Resistance, Diabetic Wound Infection

1. Introduction

The emergence of microbial resistance to multiple antimicrobial agents has become a significant global concern, resulting in adverse effects on the patients (Jindal, Pandya & Khan, 2015). Multi-drug resistance (MDR) is defined as the resistance of microorganisms to more than two antibiotic groups. The emergence of MDR causes increased rates morbidity and mortality. In addition, the increased costs of treatment have limited the effectiveness of the existing antibiotics which cause failure of treatment (Levy & Marshall, 2007; Lin et al., 2015). Hospital-acquired infections caused by MDR bacteria are a major challenge for clinicians. *P. aeruginosa* and *Staphylococcus aureus* are the most widespread MDR bacteria (Walsh & Amyes, 2004).

The deficient action or secretion of insulin is a clinical syndrome associated with diabetes mellitus (DM). In the 21st century, DM is one of the most emerging threats to health. It is expected that the number of DM persons will be 380 million in 2025 (Atkins & Zimmet, 2010). A number of people with diabetes worldwide estimated at 131 million in 2000 and expected to reach 366 million by 2030 (Clayton & Elasy, 2009). In the last decade, the prevalence of diabetes is dramatically tripled in some Arab countries (Al-Wahbi, 2006). International Diabetes Federation (IDF) recorded more than 7.8 million diabetic cases in Egypt in 2015 (IDF, 2015).

The resistance originated from indiscriminate exposure to antibiotics imposed microorganisms to have a superior ability to stay alive even under the effect of the strongest antibiotics (Fagon et al., 2000). Therefore, it is the necessary time for novel natural antimicrobials with novel mechanisms of action to diminish the problems caused by increased MDR percentages (Paul, Prasad & Sah, 2011). The high risk associated with the use of existing

synthetic antibiotics has promoted research to screen medicinal plants and their extracts as herbal sources of natural antimicrobial agents against a wide range of bacterial diseases (Gram-negative and Gram-positive) including MDR species (Arellanes, Meckes, Ramirez, Torres & Luna-Herrera, 2003). The active metabolites of the herbal plants may serve as alternative folk medicines against various MDR infectious diseases (Cowan, 1999). Plant secondary metabolites and essential oils can be used as alternative remedies for the treatment of many infectious diseases.

Essential oils (EOs) are the secondary metabolite volatile liquids of aromatic plants synthesized by all plant organs such as leaves, flowers, stems (Wanners et al., 2010), seeds (Patel & Trivedi, 2015), barks (Raut, Sawant & Jamge, 2014), fruits (Vanin et al., 2014), roots (Shah, Jani, Shah, Chaudhary & Shah, 2015) and peels (Burt, 2004; Roy et al., 2012). The EOs are found in many plants families like *Fabaceae*, *Asteraceae*, *Lauraceae*, *Rutaceae*, *Aristolochiaceae*, *Lamiaceae*, *Meliaceae*, *Cupressaceae*, *Myrtaceae*, etc. Chemically, EOs are a complex mixture of many single low molecular weight compounds derived from aromatic and aliphatic aldehydes, terpenes, phenols, as well as terpenoids (isoprenoids) (Freires, Denny, Benso, Alencar & Rosalen, 2014). EOs exhibit various biological activities like antidiabetic (Abdollahi et al., 2003), antibacterial (Nair, Vaghasiya & Chanda, 2008), anti-staphylococcal activity (Mahboubi & Bidgoli, 2010), antifungal (Tian, Ban, Zeng, He, Huang & Wang, 2011), antiviral (Edris, 2007), antioxidant (Tepe, Daferera, Kmen, Polissiou & Kmen, 2004), insecticidal (Panella et al., 2005), anticancer activity (Sylvestre, Legault, Dufour & Pichette, 2005), anti-inflammatory (Silva et al., 2003) and antimycotic activities (Baptista et al., 2015).

The activity of EOs is due to their medicinal properties against the wide range of pathogens (Akthar, Degaga & Azam, 2014). However, the spectrum of EO as antimicrobial active compound depends on many factors like the source of the antimicrobial compounds, tested microbes as well as measurement conditions (Turgis, Han, Caillet & Lacroix, 2009; Rakholiya & Chanda, 2012). EOs are one of the new ways, needed for treatment, to inhibit MDR bacterial pathogens with minimal side effects (Tohidpour, Sattari, Omidbaigi, Yadegar & Nazemi, 2010) and to increase the spectrum of the antimicrobial activity (Fadli et al., 2012). So the present study aimed to evaluate the activity of some EOs as promising active antimicrobial compounds against MDRPA isolated from diabetic wound patients.

2. Material and Methods

A total of 35 clinical specimens were randomly collected from diabetic patients attending the clinics of the Tanta University hospitals, Egypt, during 2013. Specimens were collected in nutrient broth and immediately transferred to the lab of Microbiology, Faculty of Science at Tanta University, Egypt. Each specimen was cultured on blood agar and nutrient agar plates. Then, the colonies were subcultured on MacConkey plates. For a preliminary selection of *P. aeruginosa*, non-lactose fermenting colonies were further subcultured on cetrimide agar supplemented with 15 µg/ml nalidixic acid. The standard methods of Bergey's Manual for Systematic Bacteriology were used for identification of the recovered isolates (Krieg & Holt, 2001). The isolates were confirmed with API 20E (bioMerieux) identification kits. The identified isolates were preserved in 15% glycerol at –20 °C for further experiments.

2.1 Antimicrobial Activity Testing

2.1.1 Susceptibility Testing

The tested isolates were cultivated on *Pseudomonas* isolation Agar (PIA) medium and incubated at 37 °C for 18 h. A suspension was prepared using few separate colonies for each isolate in 1-2 ml of normal saline. Each suspension was diluted using sterile normal saline to obtain cell count of about 10^7 CFU/ml using standard turbidity (corresponding to 0.5 McFarland tube). One hundred microliters of each of the previous suspensions were dropped on the center of two well-dried plates of Mueller-Hinton agar and was then spread homogeneously using a sterile cotton swab and left to dry for 15 min before incubation at 37 ºC for 18 h. Susceptibility testing was carried out with the use of the disk-diffusion method (a modified Kirby–Bauer method). Clinical and Laboratory Standards Institute was used for the interpretation of results (CLSI, 2014). The following antibiotics (oxide) being used against all tested *P. aeruginosa* isolates: Ampicillin (10 µg), Amoxicillin (25 µg), Piperacillin (100 µg), Amoxycillin/Clavulanic acid (20/10 µg), Cefoperazone/Sulbactam (75/30 µg), Piperacillin/tazobactam (100/10 µg), Ceftazidime (30 µg), Cefepime (30 µg), Ceftriaxone (30 µg), Cefoperazone (10 µg), Imipenem (10 µg), Aztreonam (10 µg), Vancomycin (30 µg), Ciprofloxacin (5 µg), Nalidixic acid (30 µg), Tetracycline (30 µg), Co-trimoxazole (Sulphamethoxazole/Trimethoprim) (25 µg), Chloramphenicol (30 µg), Gentamicin (10 µg), Amikacin (30 µg), Neomycin (30 µg),

Streptomycin (10 µg), Tobramycin (10 µg), Kanamycin (30 µg), Colistin Sulphate (10 µg). Multiple antibiotic resistance (MAR) index was calculated (Olayinka, Olayinka & Onile, 2009). Multi-drug resistant *P. aeruginosa* (MDRPA) isolates were selected based on their antibiogram patterns according to Rossolini & Mantengoli (2005) who revealed MDRPA as resistance to at least three of the six drugs, including piperacillin, imipenem amikacin, gentamicin, ciprofloxacin and ceftazidime.

2.1.2 Essential Oils

A commercial EOs from garlic (*Allium sativum*), camphor (*Cinnamomum camphora*), cinnamon (*Cinnamonum verum*), olibanum oil (*Boswellia carterii*), clove (*Syzygium aromaticum*), jojoba (*Simmondsia chinensis*), ginger (*Zingiber officinale*), black seed (*Nigella sativa*), green tea (*Camellia sinensis*), nutmeg (*Myristica fragrans*) and mustard (*Sinapis alba*) were purchased from local retail market and stored in dark vials at 4 ºC to investigate their antibacterial activities against selected MDRPA.

2.1.3 Antibacterial Activity Assays

The sensitivity of the selected MDRPA isolates was tested against various EOs by the modified Kirby-Bauer method on Muller-Hinton agar as mentioned above.

2.1.4 Minimum Inhibitory Concentration (MIC)

Microdilution broth method was conducted to determine the antibacterial activity of the tested EOs. Each tested EO was diluted by using ethanol and then mixed with Mueller-Hinton broth (100 µl) to obtain concentrations from 2.0 to 9.0 µl/ml. An inoculum containing $1.5x10^8$ CFU (10 µl) per well was added to a broth with various oil concentrations; one well (control) was filled with broth without oil. All mixtures were then transferred to 96-well microtiter plates. Minimal Inhibitory Concentration (MIC) was defined as the lowest oil concentration at which the visible bacterial growth was inhibited after 18 h of incubation at 37 °C.

2.2 Characterization of *Nigella sativa*

2.2.1 Gas Chromatography-Mass Spectrum (GC-MS)

The unsaponifiable fraction of the tested sample was removed by ether. Free fatty acids were obtained by addition of H_2SO_4 (2.5%) to the soapy solution. The liberated fatty acids were obtained by ether extraction and dried over anhydrous Na_2SO_4. Ethereal diazomethane solution was extracted corresponding methyl esters from the extracted fatty acids (Farag, Ahmed &

Ewies, 1986). Gas chromatography (HP 6890 Series) was conducted for fatty acid methyl esters fractionation. The TR-FAME column (Thermo 260 M142 P) with 30 m, 0.25 mm ID, 0.25 mm Film and 70% Cyanopropyl-Polysilph-phenylene siloxane capillary column. The injector Temperature was 200ºC and temperature transfer line 250ºC. The carrier gas was He (1.5 ml/min) at ionization energy (70 eV). One microliter of the injected sample was 5 µl/l ml solvent. With reference to authentic reference compound and probability, merges search libraries software of NIST.11.L, Wiley7n.l, and Pest. l, the relative retention times and mass spectra were determined to identify different constituents of the tested material (Adams, 2001).

2.2.2 FT-IR Analysis

The FT-IR spectrum of *N. sativa* was obtained separately using Bruker-Tensor-27-FT-IR spectrometer (Germany) at the Tanta University Central Lab. FT-IR was used to determine the functional groups with the help of correlation charts. The wave number region for analysis of *N. sativa* oil was 5000-200 cm^{-1}.

2.3 Statistical Analysis

Results are presented as the mean ± standard deviation (SD) from three replicates. The statistical analysis was carried out using SAS (v 6.12). Data obtained were analyzed statistically to determine the degree of significance using one-way analysis of variance (ANOVA) at probability level $P \leq 0.05$.

3. Results and Discussion

MDRPA isolated from wounds remains the leading pathogen in this type of infections (Lari, Bahrami & Alaghehbandan, 1998). Once *P. aeruginosa* is established in the hospital environment, it can persist for long period of time, posing as MDR nosocomial infection risk for patients being treated there (Douglas, Mulholland, Denyer & Gottlieb, 2001; Edwards & Greenwood, 2003). The investigated *P. aeruginosa* presented a resistance profile comprising resistance towards most of the common antibiotic classes (**Table 1**). Out of 35 specimens, 11 were *P. aeruginosa* (31%) and 7 (64%) of which were MDRPA. The natural resistance mechanisms of *P. aeruginosa* to several antibiotics owing to impermeable outer membrane, β-lactamase production and inducible efflux systems (Lister, Wolter & Hanson, 2009). According to resistance patterns, *P. aeruginosa* was classified into three phenotypes (Pechere & Kohler,

1999; Strateva & Yordanov, 2009). The first phenotype pattern being resistant to some of non β-lactam antibiotics as chloramphenicol, tetracycline, quinolones, and trimethoprim as well as resistant to meropenem as one of the β-lactams. The second phenotype displays resistance to all β-lactams except carbapenems and cephems including cefepime and cefpirome. In the third phenotype resistance includes penicillins (azlocillin, piperacillin and ticarcillin), it is affected more than resistance to cephalosporins. In the present study, the resistance pattern displayed by MDRPA is in general agreement with the first phenotype.

The resistance of tested MDRPA to the 4th generation cephalosporins (cefepime) was 100%. The result was in accordance to Satti, Abbasi, Qumar, Khan & Hashmi (2011) who found that 71% of MDRPA were resistant to cefepime. Mizuta et al. (2006) recorded that 87% and 84% of *P. aeruginosa* were sensitive to ceftazidime and amikacin, respectively. These results were disagreeing partially with our results which showed 100% resistance to ceftazidime but accepted for amikacin (42.9%) resistance. In our study, 83.3% of MDRPA were resistant to aztreonam. This antibiotic has an excellent activity against *Pseudomonas* species but with limited activity against MDRPA (Douglas et al., 2001). Although a high resistance to ciprofloxacin might be due to the widespread prescribing of fluoroquinolones in empirical therapy for *Pseudomonas* infections, the present results were disagreeing with other studies (Hsu, Okamoto, Murthy & Wong-Beringer, 2005; Kaye et al., 2006). Piperacillin showed resistant (42.9%) to MDRPA isolates, this finding disagrees to another study in which the isolates remained 90% susceptible to piperacillin (Walton, Villarreal, Herndon & Heggers, 1997). Carbapenems (Imipenem, Meropenem) are useful in the treatment of some cases of MDRP (Douglas et al., 2001). In this study, the resistance of *P. aeruginosa* against imipenem was 42.9%. This result was in accordance with Tam et al. (2010). The sensitivity of MDRPA to colistin sulfate might be due to the less frequent use of this drug in the general practice

Table 1: *Incidence of Antimicrobial Resistance among MDR Pseudomonas Aeruginosa Isolates*

Antimicrobial agent	No. (%) of resistant isolates
β-LACTAMS	
Amoxicillin (AX)	7(100%)
Amoxycillin/Clavulanic acid (AMC)	7(100%)
Ampicillin (AMP)	7(100%)
Aztreonam (ATM)	7(100%)
Ceftazidime (CAZ)	7(100%)
Cefepime (FEP)	7(100%)
Ceftriaxone (CRO)	7(100%)
Cefoperazone (CFP)	4(57.1%)
Cefoperazone/Sulbactam (CES)	3(42.9%)
Imipenem (IPM)	3(42.9%)
Piperacillin (PRL)	3(42.9%)
Piperacillin/Tazobactam (TPZ)	3(42.9%)
AMINOGLYCOSIDES	
Amikacin (AK)	3(42.9%)
Gentamicin (CN)	3(42.9%)
Kanamycin (K)	7(100%)
Neomycin (N)	7(100%)
Streptomycin (S)	5(71.4%)
Tobramycin (TOB)	4(57.1%)
FLUOROQUINOLONES	
Ciprofloxacin (CIP)	1(14.3%)
Nalidixic acid (NA)	7(100%)

FOLATE PATHWAY INHIBITORS (Sulphonamide)	
Co-trimoxazole (Sulphamethoxazole/Trimethoprim) (SXT)	7(100%)

Table 1 *(cont.)*

Antimicrobial agent	No. (%) of resistant isolates
TETRACYCLINES	
Tetracycline (TE)	6(85.7%)
PHENICOLS	
Chloramphenicol (C)	5(71.4%)
LIPOPEPTIDES	
Colistin Sulphate (CT)	3(42.9%)
GLYCOPEPTIDES	
Vancomycin (VA)	7(100%)

Table 2: *Resistance Patterns and MAR index of MDR Pseudomonas Aeruginosa Isolates*

Pattern code		Antimicrobial resistance pattern*	No. of isolates exhibit this pattern	MAR index
A	A1	TOB,N,CFP,C**, X,Y,Z**	1 (*P. auerginosa* 1)	0.6
	A2	TE,CES,TPZ**, X,Y,Z**	1 *(P. aeruginosa* 2)	
	A3	S,TE,C**, X,Y,Z**	1 (*P. aeruginosa* 3)	
B	B1	TOB,S,TE,CES,TPZ,C,CT**, X,Y,Z**	1 *(P. aeruginosa* 7)	0.76
C	C1	IMP,CN,TOB,S,AK,CFP,TE,CIP,C,PRL**, X,Y,Z**	1 (*P. aeruginosa* 4)	0.88
	C2	IMP,CN,TOB,S,AK,CFP,TE,C,PRL,CT**, X,Y,Z**	1 (*P. aeruginosa* 5)	
	C3	IMP,CN,S,AK,CFP,TE,CES,TPZ,PRL,CT**, X,Y,Z**	1 (*P. aeruginosa* 6)	

MAR: Multiple antibiotic resistances**;**

X: Resistance pattern include AMP AX AMC and CAZ;

Y: Resistance pattern include FEB CRO ATM and N;

Z: Resistance pattern include K NA SXT and VA;

***AMP**: ampicillin; **AX**: amoxicillin; **PRL**: piperacillin; **AMC**: amoxicillin/clavulanic acid; **TPZ**: piperacillin/tazobactam; **CAZ**: ceftazidime; **FEP**: cefotaxime; **CRO**: ceftizoxime; **CFP**: cefoperazone; **IPM**: imipenem; **ATM**: aztreonam; **CES:** cefoperazone/sulbactam; **CIP**: ciprofloxacin; **SXT**: co-trimoxazole; **TE**: tetracycline; **C**: chloramphenicol; **CN**: gentamicin; **AK**: amikacin; **N**: neomycin; **S**: streptomycin; **TOB**: tobramycin; **K**: kanamycin; **CT**: colistin sulphate; **VA**: vancomycin; **NA**: nalidixic acid.

Seven heterogeneous resistance patterns were recorded (**Table 2)** which explain the ability of these MDRPA isolates to use several mechanisms of resistance. Further, isolates that were resistant to one class of antibiotic groups were also resistant to at least one other class (Finlayson & Brown, 2011). Multiple antibiotic resistance index (MAR) is helpful in analyzing health risk, and to check the antibiotic resistance (Riaz, Faisal & Hasnain, 2011). In the present study, the MAR index analysis revealed that all MDRPA had MAR index value larger than 0.2, which indicate that this resistance was originated from the environment of antibiotics. (Krumpernam, 1983; Paul, Bezbarauh, Roy & Ghosh, 1997; Tambekar, Dhanorkar, Gulhane, Khandelval & Dudhance, 2006; Olayinka et al., 2009).

Table 3: *Antibacterial Activity of Selected EOs against MDRPA*

Essential oil	PA1	PA2	PA3	PA4	PA5	PA6	PA7
Black seed	7.6 ± 0.2^{a}	0.0 ± 0.0^{b}	24.7 ± 0.3^{d}	7.2 ± 0.3^{b}	7.8 ± 0.2^{c}	6.8 ± 0.3^{a}	8.6 ± 0.6^{c}
Camphor	7.3 ± 0.6^{a}	6.8 ± 0.3^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	6.7 ± 0.3^{a}	7.9 ± 0.2^{a}	7.2 ± 0.3^{a}
Cinnamon	0.0 ± 0.0^{b}	6.7 ± 0.3^{a}	0.0 ± 0.0^{a}	6.8 ± 0.3^{b}	0.0 ± 0.0^{b}	7.1 ± 0.2^{a}	0.0 ± 0.0^{b}
Clove	7.1 ± 0.2^{a}	0.0 ± 0.0^{b}	7.2 ± 0.3^{b}	0.0 ± 0.0^{a}	6.7 ± 0.3^{a}	7.2 ± 0.3^{a}	0.0 ± 0.0^{b}
Garlic	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	8.8 ± 0.3^{c}	0.0 ± 0.0^{a}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	7.1 ± 0.2^{a}
Ginger	6.8 ± 0.3^{a}	7.2 ± 0.3^{a}	7.1 ± 0.2^{b}	0.0 ± 0.0^{a}	7.2 ± 0.3^{ac}	7.2 ± 0.3^{a}	7.1 ± 0.2^{a}
Green tea	0.0 ± 0.0^{b}	7.5 ± 0.5^{ac}	0.0 ± 0.0^{a}	7.2 ± 0.3^{b}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	7.2 ± 0.3^{a}
Jojoba	7.2 ± 0.3^{a}	0.0 ± 0.0^{b}	6.8 ± 0.3^{b}	0.0 ± 0.0^{a}	0.0 ± 0.0^{b}	6.8 ± 0.3^{a}	7.1 ± 0.2^{a}
Mustard	8.8 ± 0.3^{c}	0.0 ± 0.0^{b}	0.0 ± 0.0^{a}	6.7 ± 0.3^{b}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	6.7 ± 0.3^{a}
Nutmeg	7.8 ± 0.3^{a}	8.0 ± 0.5^{c}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	6.7 ± 0.3^{a}	7.2 ± 0.3^{a}	0.0 ± 0.0^{b}
Olibanum	8.2 ± 0.3^{ac}	0.0 ± 0.0^{b}	9.2 ± 0.6^{c}	0.0 ± 0.0^{a}	7.3 ± 0.3^{a}	7.1 ± 0.2^{a}	0.0 ± 0.0^{b}

Values are mean inhibition zone (mm) ± S.D of three replicates, 0= no zone of inhibition.

Values with the same letter in the same column are insignificant ($p \leq 0.05$).

Currently, EOs displayed variable activities against MDRPA. *N. sativa* exhibited the highest antibacterial activity. Meanwhile camphor, ginger and olibanum oils displayed a moderate activity whereas cinnamon, clove, garlic, green tea, jojoba, mustard and nutmeg exhibited weak effect against the test MDRPA (**Table 3**). El-Shouny and Magaam (2009) recorded a strong activity of *Thymus vulgaris* EO against MDRPA. Furthermore, Pereira,

Sumita, Furlan, Jorge & Ueno (2004) showed the activity of sage oil against various bacterial pathogens which exhibited 100% efficiency against *K. pneumoniae*, 75% against *Morganella morganii*, and no activity against *P. aeruginosa*.

MDRPA3 was found to be sensitive to *N. sativa* oil at MIC (2.0 µl/ml). Sun et al. (2015) reported the activity of *Dictamnus angustifolius* EO extracted from root against *P. aeruginosa*. Burt and Reinders (2003) reported the antibacterial activity of oregano and light thyme as potent EOs against *E. coli* O157:H7.

The antimicrobial activity of *N. sativa* oil was recommended against several MDR bacteria isolated from wound infections and may be used topically in susceptible cases (Salman, Khan & Shukla, 2008; Gerige, Gerige & Rao, 2009; Falsafi et al., 2015). Mechanism of action of EOs varied from a different part of the plant and various active constituent present in them. Different compounds present in the EOs have a different mode of action and different biological effects, i.e. antibacterial, antifungal, antiviral and cytotoxicity effects. There are some commonly accepted mechanisms of action of essential oil in the antimicrobial interaction. The mechanism of action of essential oil involves so many targets in the cell due to a large number of active constituent. The antimicrobial actions of EOs were concluded by Lopez, Palou & Malo (2007) to include inactivation of microbial enzyme, cell membrane leakage, increasing the permeability of the cell membrane as well as inhibition of a common biochemical pathway. EOs may disrupt the structure of different fatty acids, polysaccharides, and phospholipids layers present in the cell wall and cytoplasmic membrane (Longbottom, Carson, Hammer, Mee & Riley, 2004). EOs can cause disruption of the membrane in microorganisms by the action of lipophilic compounds in the oils and thereby impart antibacterial activity (Sahoo, Mulla, Ansari & Mohandass, 2012). In the current study, GC/MS analysis of *N. sativa* identified 4 significant peaks linoleic acid, methyl ester was the major compound (34.59%) followed by palmitic acid, methyl ester (32.98%), phthalic acid, dioctyl ester (17.39%) linolelaidic acid, methyl ester (4.26%) as shown in **Table 4**. Much attention has been attracted to fatty acids and derivatives due to no toxicity in low concentrations as well as their activities as antimicrobial agents (Zhang et al., 2012). Linoleic acid is the key ingredients of antimicrobial food additives and in some antibacterial herbs (Zheng et al., 2005). Phthalates are reported to have antimicrobial and other pharmacological activities (Srinivasan, Sharanappa, Leela, Sadashiva & Vijayan, 2009).

The EOs are complex mixtures of a large number of molecules, the synergistic antimicrobial activity of EOs are the result of a synergism of all molecules. The main constituents of certain essential oils are terpineol, monoterpene, eugenol, safrole, thymol, carvacrol, eucalyptol, geraniol, citronellol, limonene, cinnamaldehyde which are responsible for various pharmacological activities.

Table 4: *GC-MS Antimicrobial Compounds of Black Seed Oil*

ID#	Retention Time	Compound Name
1	28.381	Palmitic acid, methyl ester
2	29.250	n-Hexadecanoic acid
3	32.024	Linoleic acid, methyl ester
4	32.580	9,12-Octadecadienoic acid, methyl ester(Z,Z)
5	33.200	Octadecanoic acid, 2-oxo, methyl ester
6	34.662	linolelaidic acid, methyl ester
7	37.719	Phthalic acid, dioctyl ester
8	38.065	Eicosane
9	39.600	Tetradecenal
10	42.953	Tetratetracontane
11	45.329	Heneicosane
12	48.248	Hexatriacontane

These major components have a different mode of action and activity is dependent on the concentration of a component, which combination of the component have been used and which main molecules are present at the highest levels in the mixture (Betoni, Mantovani, Barbosa, Stasi & Junior, 2006). The most important feature is the distribution of EOs in the cell which determines the different type of reactions and biological responses induced in the cell. It is also possible that sometimes the EOs themselves may not possess compounds that show antimicrobial activity but can operate in synergy with antibiotics or other plant extracts and are able to

sensitize the microorganisms which are ineffective to antibiotics (Betoni et al., 2006; Guerra et al., 2012).

Recently, FT-IR is one of the most applicable methods used to identify the chemical structures of a tested sample (Gough, Zelinski, Wiens, Rak & Dixon, 2003; Vlachos et al., 2006). In the present study FT-IR analysis was performed in *N. sativa*. The results of FT-IR peak values and functional groups were represented in **Figure1** and **Table 5**.

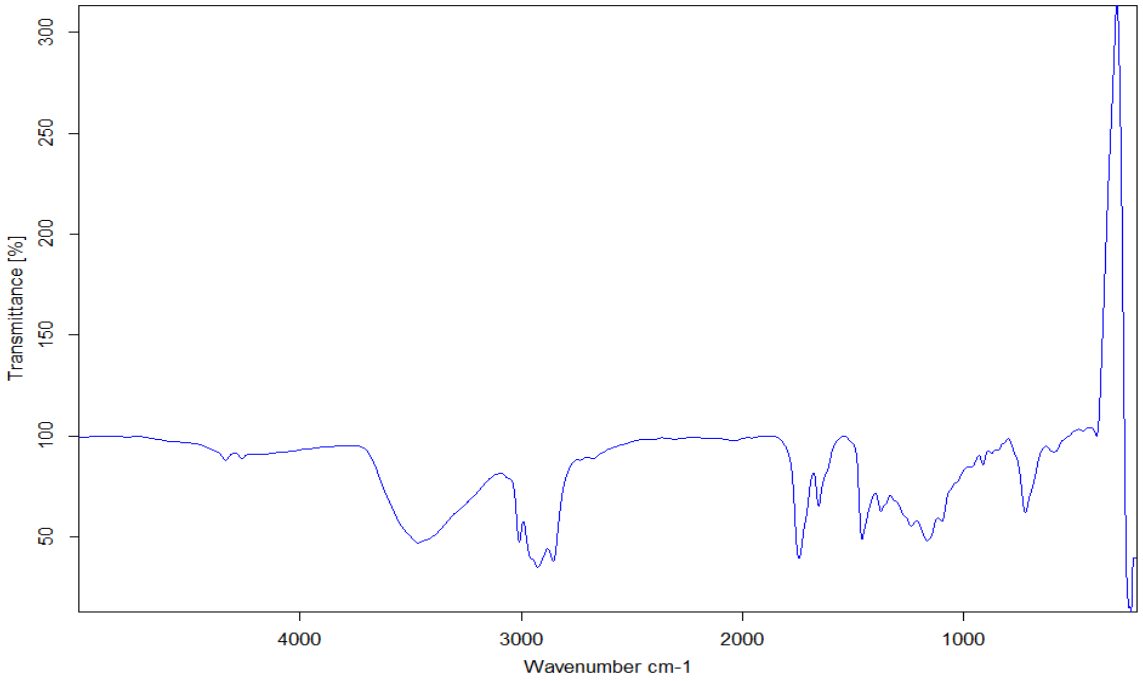

Figure 1: *FT-IR analysis of Black Seed Oil*

Table 5: *Functional Group Profiles of Black Seed Oil by FT-IR analysis*

Functional group	Range of wave number (cm^{-1})	Presence in black seed oil
Acetylenic group (C≡C)	2000-2250	Absent
Aldehyde group (CHO)	2850-2900	Present
Aliphatic saturated hydrocarbon chains (CH_3, CH_2, CH)	2850-2980	Present
Aliphatic unsaturated hydrocarbon double bonds (-C=C-)	1500-1600	Present
Amino group (NH_2)	3200-3400	Absent
Aromatic hydrocarbon ring	3100-3200	Absent

Carbonyl group (-C=O)	1700-1750	Present
Cyano group (C≡N)	2000-2250	Absent
Hydroxyl group (OH)	3300-3500	Present

The result profile showed the presence of functional groups like hydroxyl, alkanes, aldehyde, carbonyl and alkenes and there are no peaks for toxic cyanic or acetylenic groups which were confirmed by Ali & Blunden (2003) that they studied the toxicological properties of *N. sativa* and revealed that it has a low level of toxicity.

5. Conclusion

This study revealed that the pathogenic MDRPA isolated from diabetic wound infections were multi-resistant to the commonly used antibiotics, and these bacterial isolates were effectively inhibited by medicinal oils especially black seed oil. Therefore, these natural products can be considered as potential therapeutic agents for the eradication of bacteria responsible for nosocomial infections.

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