Nigella sativa A CULINARY HERB WITH ANTIBACTERIAL ACTVITY

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Abstract. *Nigella sativa* a culinary herb with antibacterial activity. Currently, the alarming increase of urinary tract infections across the world is in conjunction with the increased resistance of pathogenic bacteria to existing antibiotics. Medicinal plants are an inexhaustible source of compounds with bactericidal or bacteriostatic activity. It was evidenced that a less studied plant, *N. sativa* Linne 1753 mainly used as spice in Arab cuisine has antibacterial properties. The study was done on a lot of 40 of Gram negative bacteria with resistance to last generation antibiotics: cephalosporins, carbapenems, fluoroquinolones, which have been isolated from urinary infections from patients hospitalized. The hydroethanolic concentration of *N. sativa* extract had values between 500 and 61.25 g/ml.

Keywords: *Nigella sativa*, MDR bacteria, antibacterial activity.

Rezumat. *Nigella sativa* o plantă culinară cu activitate antibacteriană. Creşterea alarmantă a infecţiilor urinare la nivel mondial este coroborată cu rezistenţa crescută a bacteriilor patogene la antibioticele existente în prezent. Plantele medicinale reprezintă o sursă inepuizabilă de compuşi cu acţiune bactericidă sau bacteriostatică. O plantă mai puţin studiată, *N. sativa* Linne 1753, folosită în special ca şi condiment în bucătăria arabă, s-a dovedit a avea proprietăţi antibacteriene. Studiul de faţă a fost făcut pe un lot de 40 bacterii Gram negative cu rezisteţă la antibiotice de ultimă generaţie, cefalosporine, carbapeneme, fluoroquinolone, care au o fost izolate din infecţii urinare provenite de la pacienţi spitalizaţi. Concentraţia hidroetanolică a extractului de *N. sativa* a avut valori cuprinse între 500 şi 61.25 g/ml.

Cuvinte cheie: *Nigella sativa*, bacterii MDR, activitate antibacteriană.

INTRODUCTION

Nigella sativa L. (Ranunculaceae) is a plant where her properties are developing due to a very wide pharmacological potential. *N. sativa* (also known as black caraway or Negril) is native in Southern Europe, North Africa and Southwest Asia and is cultivated in many countries all over the world like Middle Eastern Mediterranean region, South Europe, India, Pakistan, Syria, Turkey, Saudi Arabia. Generally, the spices antioxidant properties are of great interest in terms of the impact of oxidative changes and lowering LDL cholesterol which is responsible for the occurrence of diseases (OPRICA, 2011). Recent studies have revealed the spectrum of action of these seeds: diuretic, antihypertensive, antidiabetic, anticancer and analgesic, immunomodulatory, antimicrobial, anthelmintic, analgesic and anti-inflammatory, antispasmodic, bronchodilator, gastroprotective, hepatoprotective, antioxidant properties and renal protection. Most ofthe therapeutic properties of this plant are due to the presence thymoquinone (TQ), which is a main active chemical component of the essential oil. Black seeds are also used in the food products, flavorings, because it has a very low toxicity (AHMED et al., 2013). Bacterial urinary tract infections are a major problem worldwide due to the resistance of bacteria to antibiotics empirical treatments, by developing new virulence factors. Recurrent infections are due for the most part of the same uropathogenic agent. The treatment of recurrences is always more complicated when being accompanied by severe complications. UTI recurrences and relapses are frequent, especially in patients with MDR bacterial infections. The antimicrobial resistance has become a major public health problem worldwide, and several studies have reported an increasing incidence of infections by MDR bacteria in both immunocompetent and immunocompromised hosts (BODRO et al., 2015). This study represents a new approach to eradicate MDR bacteria isolated from urinary infections of immunocompromised patients. For this purpose, we used the hydroethanolic extract derived from the *N. sativa*.

MATERIAL AND METHODS

1. Isolation and analysis of MDR bacteria

Strains were isolated from urinary tract infections from hospitalized patients at Theodor Burghele Hospital Bucharest. Phenotypic resistance to antibiotics was determined by disk diffusion method.

2. Preparation of the extract and analysis of the compounds

N. sativa seeds were harvested in September 2015 on the culture during the respective year. The hydroethanolic extract was prepared by macerating 30 g seeds powder in 50 ml ethyl alcohol and 20 ml distilled water for 10 days and after it was purified by Watthmann filter paper no. 1. The extract thus obtained it was introduced into a rotovaporator for 10 minutes at a speed of 125 rpm and stored in an amber glass container at 4°C. This was the stock solution from which had been made binary dilutions. The analysis compounds of the ethanolic extracts was made using TLC (Thin-Layer Chromatography method). All standard compounds and reagents ferulic acid, gallic acid, chlorigenic acid, quercetin, rutin, kaempferol, diphenylborinic acid aminoethylester, polyethylene glycol 400 (macrogol) were purchased from (Darmstadt, Germany). The Silicagel 60F254 HPTLC plates were purchased from Merck (Darmstadt, Germany).

3. Determination of antimicrobial activity of hydroethanolic extract of *N. sativa*

Quantitative determination of antimicrobial activity and establishing minimum inhibitory concentration (MIC) was made by the method of serial microdilution in a liquid medium BHI (Hearth Infusion Broth) in deepwell plates 96 (Eppendorf tubes of 550 ml) following the protocol written to a recent article (ROMAN et al., 2015).

RESULTS AND DISCUSSION

Following the analysis of antibiotic resistance via disk diffusion method, showed that the strains studied had resistance to multiple antibiotics. In table 1 is shown the screening of antibiotic resistance.

Table 1. The antibiotic resistance of strains studied.

No.	strain	S (sensitivity)	R (resistance)
1	*K. pneumoniae*$_{I1}$	CL IMI CM	AMC CAZ FEP LEV SXT TZP
2	*K. pneumoniae*$_{I2}$	CL IMI ERT TZP	AMC CAZ FEP CM LEV SXT
3	*K. pneumoniae*$_{I3}$	CL ERT IMI LEV TZP	AMC CAZ FEP CM SXT
4	*P. mirabilis*$_{I1}$		AMC CAZ FEP CIP CM IMI ERT STX TZP
5	*E. coli*$_{I2}$	CL FOT IMI TZP	AMC CAZ FEP
6	*E. coli*$_{I3}$	CL FOT IMI TZP	AMC CAZ FEP CIP CM SXT
7	*P. aeruginosa*$_{I4}$	CL	CAZ FEP CIP CM TZP ATM IMI
8	*A. baumannii*$_{I5}$		AMC CAZ FEP CIP CM IMI SXT TZP
9	*K. pneumoniae*$_{I6}$	CL CM	AMC CAZ FEP CIP CM ERT IMI SXT TZP
10	*K. pneumoniae*$_{I10}$	ERT IMI CL SXT	AMC CAZ FEP CIP TZP
11	*P. aeruginosa*$_{I11}$	CL	CAZ FEP CM CIP ATM TZP IMI MEM
12	*K. pneumoniae*$_{I12}$	ERT MEM	AMC CAZ FEP CIP CM TZP
13	*E. coli*$_{I13}$	CL ERT MEM FOT	AMC CAZ FEP CM CIP SXT
14	*K. pneumoniae*$_{I14}$	CL	AMC CAZ FEP CIP CM ERT MEM TZP SXT
15	*K. pneumoniae*$_{I15}$	CL	AMC CAZ FEP CIP CM ERT MEM TZP SXT
16	*E. coli*$_{I16}$	FOT CM MEM TZP	AMC CAZ FEP CIP SXT
17	*E. coli*$_{I17}$	CIP FOT CM IMI SXT	AMC CAZ FEP TZP
18	*K. pneumoniae*$_{I19}$	CL	AMC CAZ FEP IMI CIP CM SXT TZP
19	*P. aeruginosa*$_{I20}$	CL	CAZ FEP CIP CM IMI MEM TZP
20	*A. baumannii*$_{I22}$	CL	AMC CAZ FEP CM IMI MEM SXT TZP ATM
21	*K. pneumoniae*$_{I23}$	CL CM	AMC CAZ FEP ERT MEM CIP SXT TZP
22	*P. aeruginosa*$_{I24}$	CL	IMI MEM ATM CM CIP CAZ FEP TZP
23	*A. faecalis*$_{I25}$		CL IMI MPM ATM CM CIP CAZ FEP TZP
24	*K. pneumoniae*$_{I26}$	CL LEV ERT MEM CM	AMC CAZ FEP TZP

Acinetobacter baumannii, *Pseudomonas aeruginosa* and *Algaligenes faecalis* are the most frequently bacteria isolated in nosocomial infections. The enhance resistance to aminoglycosides, cephalosporins, quinolones, penicillins, monobactams and imipenem of this bacteria was reported in numerous articles. Colistin (Polimixin E) is a polypeptide antibiotic group discovered in 1947–1948. Once the second- and third generation cephalosporins have become available, the use of colistin was dropped, mainly because of toxicity. The mechanism of action of colistin is not very clear, it is most likely a direct activity at the cell membrane (LEVIN et al., 1999). In vitro, the colistin manifested an activity against all Gram negative bacteria, with the exception of three bacteria *(Algaligenes faecalis*$_{I25}$*, A. baumannii*$_{I5}$ and *P. mirabilis*$_{I1}$*)* that exhibited resistance to all antibiotics tested.

The fingerprints obtained by detection at different wavelengths after derivatization allowed the observation of diverse features of the same chromatographic separation offering comprehensive information. It can be seen from chromatograms that using either visible detection or UV detection at 254 nm is not sufficient for extract fingerprinting. The most complete information about extract constituents was obtained by fluorescence detection. The chromatograms clearly indicate that *N. sativa* extract contains quercetin and kaempferol (Fig. 1). Quercetin and kaempferol are two flavonols, frequently present in the form of glycosides (OPRICA, 2016) whose antioxidant and antibacterial activities have been shown in several articles. Thus, the antibacterial activity of *Allium cepa* is due to its skin as it has a high content of free and glycosidically bonded quercetin and oxidized quercetin derivatives (RAMOS et al., 2006). *Bryophyllum pinnatum* is a plant native to Madagascar, also known asthe “life plant“, “resurrection plant“ or “goodluck“, has a wide range of medicinal uses, inclusively very severe infections. The major compound of this plant is kaempferol to which it assigns the antibacterial properties (TATSIMO et al., 2012). Flavonols are not essential to plant growth but have a major role in their defense of microorganisms. This explains their antibacterial activities. Flavonoids may act through inhibiting cytoplasmic membrane function (PAIVA et al., 2010).

The sequencing of standard plates and extract compounds: 1-ferulic acid, 2-chlorogenic acid, 3-quercetin, 4-hydroethanolic extract of *N. sativa*, 5-rutin, 6-kaempferol.

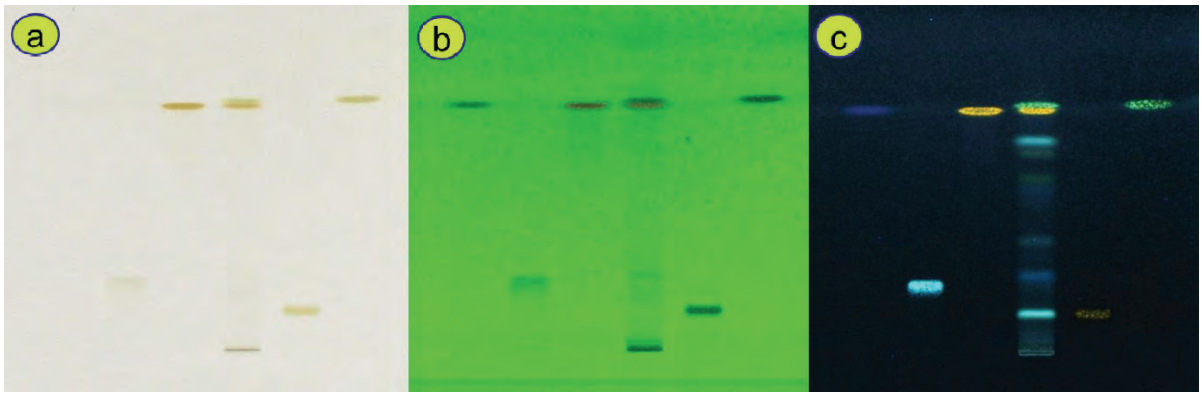

Figure 1. Chromatographic fingerprint viewed at at visible light (a), at UV 254 nm (b) and at UV 366 nm(c).

Qualitative and quantitative evaluation of the antimicrobial activity of clove extracts

The qualitative antibacterial testing of *N. sativa* hydroethanolic extract showed the antibacterial properties of the compounds isolated from *N. sativa* extract. In Fig. 2 is shown the antibacterial activity qualitative by disc diffusion method adapted.

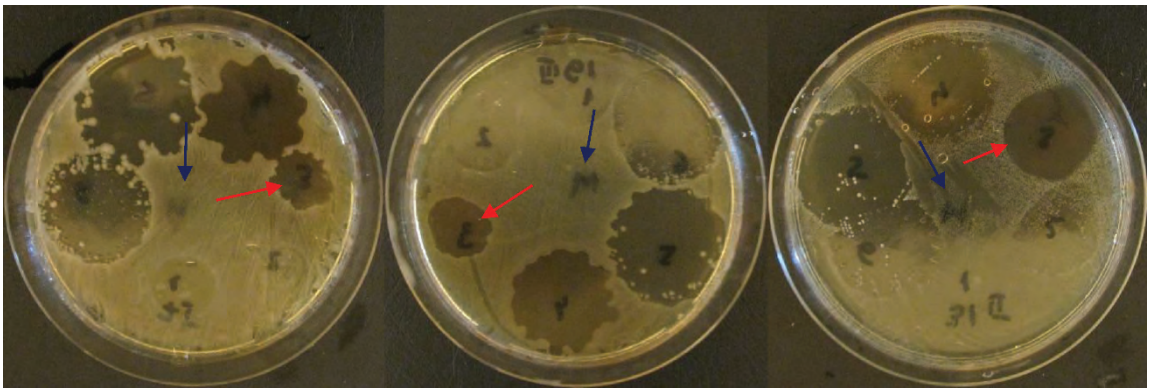

Figure 2. Qualitative testing of susceptibility to hydroethanol extract of *N. sativa*. Red arrows indicate the inhibition of bacterial growth of extract herb and the blue arrows, no inhibition of the negative control (ethanol in this example).

The minimum inhibitory concentration (MIC) by the method of binary dilutions in 96 wells plates ranged between 31.25 and 500 mg / ml. In table 2 are shown shows the values of MIC read at an absorbance of 620 nm.

Table 2. MIC of hydroethanolic extract of *N. sativa* observed at spectrophotometer at 620 nm.

Strain	**Extract concentration of *N. sativa***							
	500	250	125	62.5	31,25	15.625	7.831	3.9
K. pneumoniae$_{I1}$	0.059	0,079	0.203	0.298	0.301	0.501	0.529	0.578
K. pneumoniae$_{I3}$	0.061	0.098	0.173	0.301	0.324	0.509	0.579	0.581
E. coli$_{I2}$	0.058	0.099	0.208	0.312	0.431	0.517	0.581	0.592
E. coli$_{I3}$	0.059	0112	0.217	0.378	0.504	0.522	0.576	0.581
P. aeruginosa$_{I4}$	0,063	0.089	0.198	0.301	0.512	0.539	0.578	0.589
A. baumannii$_{I5}$	0.064	0,121	0.238	0.334	0.499	0.552	0.593	0.591
K. pneumoniae$_{I6}$	0.059	0.118	0.279	0.321	0.516	0.571	0.586	0.589
K. pneumoniae$_{I10}$	0.065	0.109	0.234	0.317	0.527	0.583	0.592	0.596
P. aeruginosa$_{I11}$	0.071	0.171	0.197	0.276	0.531	0.591	0.585	0.589
K. pneumoniae$_{I12}$	0.077	0.169	0.173	0.281	0.517	0.586	0.584	0.587
E. coli$_{I13}$	0.081	0.093	0.204	0.286	0.537	0.569	0.569	0.578
K. pneumoniae$_{I14}$	0.078	0.099	0.179	0.211	0.239	0.582	0.587	0.582
K. pneumoniae$_{I15}$	0.069	0.131	0.191	0.283	0.479	0.592	0.589	0.293
E. coli$_{I16}$	0.059	0.103	0.185	0.227	0.389	0.587	0.591	0.598
E. coli$_{I17}$	0.093	0.117	0.134	0.146	0.507	0.577	0.586	0.587
K. pneumoniae$_{I19}$	0.078	0.208	0.241	0.309	0.558	0.539	0.593	0.596
P. aeruginosa$_{I20}$	0.086	0.179	0.231	0.334	0.574	0.581	0.586	0.589
A. baumannii$_{I22}$	0.077	0.111	0.143	0.157	0.398	0.594	0.593	0.588
K. pneumoniae$_{I23}$	0.089	0.207	0.271	0.369	0.423	0.562	0.578	0.593
P. aeruginosa$_{I24}$	0.096	0.301	0.328	0.418	0.509	0.586	0.592	0.595
A. faecalis$_{I25}$	0.089	0.115	0.262	0.371	0.532	0.591	0.593	0.598
K. peumoniae$_{I26}$	0.057	0.068	0.123	0.171	0.571	0.589	0.594	0.597
M^-	0.058	0.059	0.057	0.059	0.057	0.056	0.058	0.059
M^+	0.598	0.597	0.596	0.601	0.597	0,589	0.599	0.594

CONCLUSIONS

Hydroethanolic extracts of *N. sativa* can be used successfully for severe UTI infections, due, in particular to MDR Gram negative bacteria. Flavonoids were major compounds resulting from the plant's secondary metabolism to whom it has been attributed the antibacterial activity. The results obtained *in vitro* of extract of *N. sativa* against MDR bacteria confers this herb a crucial asset in the synthesis by industry for new antibacterial substances.

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