Ankara Ecz. Fak. Derg.
34 (3) 183 - 190 , 2005

J. Fac. Pharm, Ankara
34 (3) 183 -190 , 2005

ANTILISTERIAL ACTIVITY OF *BALLOTA* SPECIES GROWING IN TURKEY

ANTIBACTERIAL ACTIVITY SCREENING OF *NIGELLA* L. SPECIES GROWING IN TURKEY

TÜRKİYE'DE YETİŞEN *NİGELLA* L. TÜRLERİNİN ANTİBAKTERİYEL AKTİVİTE TARAMASI

Gamze KÖKDİL[1], Nuran DELİALİOĞLU[2], Başak ÖZBİLGİN[1], Gürol EMEKDAŞ[2]

Mersin University, Faculty of Pharmacy, Department of Pharmacognosy, Yenişehir Campus, 33169 Mersin, TURKEY

Mersin University, Faculty of Medicine, Department of Microbiology, Yenişehir Campus,33169 Mersin, TURKEY

ABSTRACT

The antibacterial activity of the petroleum ether, dichloromethane and methanol extracts obtained from seeds of eleven Nigella L. *species growing in Turkey have been screened in vitro against Gram-positive strains (Staphylococcus auereus, Enterococcus faecalis, Bacillus subtilis) and Gram-negative strains (Escherichia coli, Pseudomonas aeruginosa) by agar disc-diffusion method. Some of the extracts showed antibacterial activity.*

Keywords: *Nigella L., Ranunculaceae, Seed extracts, Antibacterial activity*

ÖZET

Türkiye'de yetişen 11 Nigella L. *türünün tohumlarından elde edilen petrol eterli, diklorometanlı ve metanollü ekstrelerin Gram-pozitif (Staphylococcus auereus, Enterococcus faecalis, Bacillus subtilis) ve Gram-negatif (Escherichia coli, Pseudomonas aeruginosa) bakterilere karşı agar disk-diffüzyon metodu ile antibakteriyal aktiviteleri taranmıştır. Ekstrelerden bazıları antibakteriyal aktivite göstermiştir.*

Anahtar Kelimeler: *Nigella L., Ranunculaceae, Tohum ekstreleri, Antibakteriyal aktivite*

INTRODUCTION

The genus *Nigella* L. (*Ranunculaceae*) includes about 20 species distributed from the Mediterranean regions to West Asia (1). It includes some important species (e.g. *N. sativa* L., *N. damascena* L. and *N. arvensis* L.) with aromatic and medicinal properties. *N. sativa*, a spicy plant, is cultivated in various parts of the world. The seeds, also known as black cumin or black caraway, are commonly used in the Middle East, Northern Africa and India as a condiment in bread and other dishes. It has been used in many Middle Eastern countries as a natural remedy for 2000 yrs. The seeds or its oil is believed to have carminative, diuretic, lactagoge and vermifuge (2). *N.sativa* seeds have a wide spectrum of medicinal properties including antimicrobial, antihelminthic, anti-inflammatory, analgesic, hypoglycemic, smooth muscles relaxant and immunostimulant activities (2, 3). In the literature, there are several reports on the antimicrobial properties of *N.sativa* and *N.damascena* seed extracts or their oils. The extracts and the oils have been reported to have a broad spectrum of activity against a number of microorganisms (2, 4-6).

The genus *Nigella* comprises about 13 species in Turkey (7, 8). One of them is *N.sativa* and known with the local name as "Çörekotu". The seeds are also used as seasoning for foodstuffs like bread and pickles among Turkish people (9). In the literature, only two species of *Nigella* growing in Turkey (*N. sativa* and *N. damascena)* have been investigated chemically (10-14). Effects of *N.sativa* seeds originating from Turkish sources have also been evaluated in animal studies (15, 16). Although antimicrobial activities of *N.sativa* and *N.damascena* seed extracts have been studied, there is no report on the antimicrobial properties of other *Nigella* species in the literature. In this report, we present study of the antibacterial activity of different polarity extracts obtained from the seeds of eleven *Nigella* species growing in Turkey. The antibacterial potentials of the petroleum ether, dichloromethane and methanol extracts against three Gram-positive and two Gram-negative bacteria strains have been investigated.

MATERIAL and METHODS

Plant Material

Mature seeds of 10 of the 13 Turkish *Nigella* species were obtained from different regions of Turkey during the seedling period. *N. fumariifolia* Kotschy and *N. stellaris* Boiss. were not investigated in this study. Because *N. stellaris* is an uncommon species that was not found during the field study and *N. fumariifolia* grows on the Islands of Rodhos and Cyprus. *N. sativa* is a commonly cultivated species and the seeds of *N.sativa* were purchased from market. Voucher specimens for each species were collected during the flowering and seedling periods and identified

by Prof. Dr. Mecit Vural and Prof. Dr. Hayri Duman (Department of Biology, Faculty of Arts and Sciences, University of Gazi, Ankara, Turkey). Herbarium vouchers were deposited in the Herbarium of the Faculty of Pharmacy, Ankara University (AEF) (Table 1).

Table 1. List of studied materials and geographic origin

Taxon	Locality, voucher*
N. orientalis L.	C_6 Maraş: Çağlayancerit, Başdervişli village, ruderal fields, 1280m, 14.06.2002, 8.07.2002, AEF 23131
N. oxypetala Boiss.	B_7 Malatya: around Inonü University Campus, fallow fields, 1500 m, 28.06.2002, 08.06.2003, AEF 23130
N. latisecta P.H.Davis	C_6 Maraş : Afşin, Yazı village, fallow fields, 1100m, 13.06.2002, 8.07.2002, AEF 23126
N. segetalis Bieb.	B_4 Aksaray : Ortaköy, fields, 900m, 30.06.2002, 1.08.2002, AEF 23134
N. arvensis L.	B_4 Aksaray : Ortaköy, fields, 900m, 07.06.2002, 30.07.2002, AEF 23135
N .damascena L.	C_6 Gaziantep : Botanic garden of the Gaziantep University, 10.07.2002, AEF 23127
N. elata Boiss.	A_2 Bursa : Uludağ University Campus, bushy places, 1100m, 26.07.2002, 25.08. 2002, AEF 23128
N. nigellastrum (L.) Willk.	C_6 Maraş: Başdervişli village, hillsides, 1400m, 14.06.2002, 9.07.2002, AEF 23132
N. unguicularis (Lam.) Spenner	C_6 Maraş: Çağlayancerit, Erince mountain, waste places, 1153m, 14.06.2002, 9.07.2002, AEF 23136
N. lancifolia Hub.-Mor.	B_4 Aksaray : Ortaköy, fallow fields, 900m, 29.05.2003, 13.07.2002, AEF 23133

* Locality information is based on the Flora of Turkey grid system (Davis, 1978, 1988). Voucher numbers are the Herbarium of the Faculty of Pharmacy, Ankara University (AEF), Turkey.

Preparation of extracts

Ground seeds of *N. orientalis*, *N. oxypetala, N. latisecta, N. segetalis, N. unguicularis, N. lancifolia, N.sativa* (50 g of each) and *N. arvensis* (14 g)*, N .damascena* (11 g), *N. elata* (16.5 g) and *N. nigellastrum* (22 g) were extracted with petroleum ether (300 ml) for 6 hours using a Soxhlet apparatus and evaporated to dryness. The yield of petroleum ether extracts were 28.0, 17.6, 20.0, 37.5, 36.5, 24.3, 22.3, 41.3, 32.9, 36.3, 32.1%, respectively. Then, the materials were extracted with dichloromethane (300 ml), for 6 hours using a Soxhlet apparatus, to give 4.2, 3.7,

2.0, 2.6, 3.1, 3.7, 1.7, 0.6, 0.6, 0.9 and 1.1 g of residue respectively. Finally, the extraction was carried out with methanol (300 ml) for 6 hours using a Soxhlet apparatus. The residues obtained by evaporation of methanol were 3.1, 2.6, 2.2, 4.8, 6.3, 2.9, 4.9, 2.9, 2.0, 1.6 and 3.4 g respectively.

Antibacterial activity

All the extracts were impregnated on empty sterilized discs having a diameter of 6 mm (Schleicher&Schuell Micro Science GmbH Dassel, Germany) in the amount of 50µl. Discs were impregnated with n-hexane, dichloromethane and 10% DMSO as negative control. Standard antibiotics discs such as Ampicillin-Sulbactam (10 µg/disc), Ofloxacin (5 µg/disc), Gentamycine

(10 µg/disc) were used as positive control. For the purpose of antibacterial evaluation five microorganisms were used. *Staphylococcus aureus* (ATCC 25923, Gram-positive) *Enterococcus faecalis* (ATCC 29212, Gram-positive) *Bacillus subtilis* (ATCC 6633, Gram-positive), *Escherichia coli* (ATCC 25922, Gram-negative), *Pseudomonas aeruginosa* (ATCC 27853, Gram-negative) microorganism strains were employed for determination of antibacterial activity. The strains obtained from Becton-Dickinson firm and Center for Culture Collections of Microorganisms, İstanbul Medical Faculty, Department of Microbiology and Clinical Microbiology, Çapa, İstanbul.

The disc-diffusion method (17) was used as a screening test for antibacterial activity. The antibacterial activity screening was performed using Mueller-Hinton II Agar (Becton Dickinson and Company, USA).

Petroleum ether and dichloromethane extracts of the seeds were dissolved in n-hexane and dichloromethane respectively, and the methanol extracts of the seeds were dissolved in 10% dimethylsulphoxide (DMSO). The extracts of *N. orientalis*, *N. oxypetala, N. latisecta, N. segetalis, N. unguicularis, N. lancifolia, N.sativa* were dissolved in the solvent to a final concentration of 100 mg/ml. The concentrations of the dichloromethane extracts of *N. latisecta*, *N. arvensis, N. damascena*, *N. nigellastrum*, *N. elata* and methanol extract of *N. elata* were 72, 30, 30, 80, 30 and 85 mg/ml. All extracts were absorbed to sterile discs in aseptic conditions to obtain 5mg/disc except for dichloromethane extracts of *N. latisecta*, *N. arvensis, N. damascena, N. nigellastrum, N. elata* and methanol extracts of *N.elata.* These extracts were absorbed to discs to obtain 3.6mg/disc, 1.5mg/disc, 1.5 mg/disc, 4mg/disc, 1.5mg/disc, 4.25 mg/disc, respectively. Discs were dried at 40°C. Dried discs were transferred onto plates containing test microorganisms. Plates were incubated at 37°C for 24 hours. After incubation periods, inhibition zones were measured and compared with those of the reference antibiotics. The experiments were carried out in duplicate.

RESULTS AND DISCUSSION

The antibacterial activities of the extracts of the title plants are shown in Table 2. The inhibition zones formed by the standard antibiotic discs (positive control) and the discs injected with n-hexane, dichloromethane and 10% aqueous DMSO (negative control) are also given in Table 2.

The petroleum ether extracts of *N. segetalis* and *N. sativa* seeds exhibited antibacterial activity against *B. subtilis* and *S. aureus*. The petroleum ether extract of *N. segetalis* showed slight antibacterial activity against *B.subtilis* whereas petroleum ether extract of *N. sativa* has good activity against *B. subtilis*. The petroleum ether extracts of *N. segetalis* and *N. sativa* showed moderate activity for *S. aureus*. Activity against *S. aureus* was found from the dichloromethane extracts of N. *segetalis*, *N. sativa and N. latisecta*, showing inhibition zones of 11-8 mm. The methanol extract of N. *sativa* were slight active against S. *aureus*. All extracts were found to be ineffective against *E. faecalis*, *E. coli* and *P. aeruginosa*.

A number of reports have been published on the antimicrobial properties of *N. sativa* extracts or its oil. Agrawal et al. reported that antibacterial effects of the essential oil showed pronounced activities even at 1:100 dilutions against several organisms that included *Staphylococcus albus, Escherichia coli*, *Salmonella typhi,* and *Vibrio cholera*. The oil was more effective against Gram-positive than Gram-negative organisms (2). It has been shown that the crude alkaloid extract and the water extract of seeds were effective against variety of organisms isolated from human patients suffering from septic arthritis, even those that were resistant to antibiotics (2). Previous work on *N. sativa* has also shown that the ethanol, ethyl acetate and water extracts of the seeds show only moderate activity against Gram-positive strains (18). Recently, the essential oil and the acetone extract of N. *sativa* were found to be potentially effective against *S. aureus* and *B.subtilis* (6). Also, the antimicrobial activity of *N. damascena* seed have previously been reported by Fico et al. (5). They used essential oil, alkaloids, phenolic compounds, butanol extract of the seeds and showed that the essential oil was effective only against Gram-positive bacteria (*S. aureus, B. cereus*). Among the extracts, the butanol was active against *P. aeruginosa and S. aureus* (5).

As a result of this study, all extracts of *N. sativa* and petroleum ether and dichloromethane extracts of *N. segetalis* have antibacterial activity especially against *S. aureus*. Petroleum ether extract of N. *sativa* was more effective against *B. subtilis* than *S. aureus*. Data of *N. sativa* obtained in our study was in accordance with previous reports (18). No antibacterial activity could be found

in the present study for the extracts obtained from *N. damascena*. This may be due to the solvent to extract the different constituents having antimicrobial activity. In conclusion, the present study shows that the extracts of *N. sativa, N. segetalis* and *N. latisecta* have antibacterial activities, especially against Gram-positive bacteria such as *S. aureus* and *B. subtilis*.

Table 2: Antibacterial activity of the extracts of *Nigella* L. species

Plant	**Extract**	**Diameter of Inhibition Zone (mm)**				
		B. subtilis	*S. aureus*	*E. faecalis*	*E. coli*	*P. aeruginosa*
N. orientalis	Petroleum ether	θ	θ	θ	θ	θ
	Dichloromethane	θ	θ	θ	θ	θ
	Methanol	θ	θ	θ	θ	θ
N. oxypetala	Petroleum ether	θ	θ	θ	θ	θ
	Dichloromethane	θ	θ	θ	θ	θ
	Methanol	θ	θ	θ	θ	θ
N. latisecta	Petroleum ether	θ	θ	θ	θ	θ
	Dichloromethane	θ	**8**	θ	θ	θ
	Methanol	θ	θ	θ	θ	θ
N. segetalis	Petroleum ether	**8**	**11**	θ	θ	θ
	Dichloromethane	θ	**11**	θ	θ	θ
	Methanol	θ	θ	θ	θ	θ
N. arvensis	Petroleum ether	θ	θ	θ	θ	θ
	Dichloromethane	θ	θ	θ	θ	θ
	Methanol	θ	θ	θ	θ	θ
N. damascena	Petroleum ether	θ	θ	θ	θ	θ
	Dichloromethane	θ	θ	θ	θ	θ
	Methanol	θ	θ	θ	θ	θ
N. elata	Petroleum ether	θ	θ	θ	θ	θ
	Dichloromethane	θ	θ	θ	θ	θ
	Methanol	θ	θ	θ	θ	θ
N. nigellastrum	Petroleum ether	θ	θ	θ	θ	θ
	Dichloromethane	θ	θ	θ	θ	θ
	Methanol	θ	θ	θ	θ	θ
N. unguicularis	Petroleum ether	θ	θ	θ	θ	θ
	Dichloromethane	θ	θ	θ	θ	θ
	Methanol	θ	θ	θ	θ	θ
N. .lancifolia	Petroleum ether	θ	θ	θ	θ	θ
	Dichloromethane	θ	θ	θ	θ	θ
	Methanol	θ	θ	θ	θ	θ
N. sativa	Petroleum ether	**15**	**12**	θ	θ	θ
	Dichloromethane	θ	**8**	θ	θ	θ
	Methanol	θ	**8**	θ	θ	θ
n-Hexane	Control	θ	θ	θ	θ	θ
Dichloromethane	Control	θ	θ	θ	θ	θ
DMSO (%10)	Control	θ	θ	θ	θ	θ
Ampicillin-sulbactam	St. A	35	33	30	20	θ
Ofloxacin	St.A	N.T	24	N.T	30	17
Gentamycine	St.A	25	20	15	20	22

θ: No inhibition zone

St.A: Standard antibiotic

N.T: Not tested

ACKNOWLEDGEMENTS

The authors thank Prof Dr. Mecit Vural and Prof.Dr. Hayri Duman (Deparment of Biology, Faculty of Arts and Sciences, University of Gazi, Ankara, Turkey) for the identification of plant materials.

REFERENCES

1. **Hegnauer, R.** *Chemotaxonomie der pflanzen*, Birkhauser verlag, Basel and Stutgart, p.43 (1973).
2. **Ali, B.H., Blunden, G.** "Pharmacological and Toxicological Properties of *Nigella sativa*" *Phytother. Res.* **17**, 299-305, (2003).
3. **Houghton, P.J., Zarka, R., Heras, B., Hoult, R.S. "**Fixed oil of *N. sativa* and derived thymoquinone inhibit eicosanoid generation in leucoytes and membrane lipid peroxidation" *Planta Med*. **61**, 33-36, (1995).
4. **Hanafy, M.S.M., Hatem, M.E.** " Studies on the antimicrobial activity of Nigella sativa seed (black cumin)", *J Ethnopharm*. **34**, 275-278 (1991).
5. **Fico, G., Panizzi, L., Flamini, G., Braca, A., Morelli, I., Tome, F., Cioni, P.L.** "Biological Screening of Nigella damascena for Antimicrobial and Molluscicidal Activities", *Phytother.Res*. **18,** 468-470 (2004)
6. **Singh, G., Marimuthu, P., Heluai, C. S., Catalan, C.** " Chemical constituents and antimicrobial and antioxidant potentials of essential oil and acetone extract of Nigella sativa seeds ", *J Sci Food Agric* **85,** 2297-2306 (2005)
7. **Davis, P.H.** *Flora of Turkey and the East Aegean Islands*, Vol.**6**, The Edinburgh University Press, Edinburgh, p.98-105, (1978).
8. **Davis, P.H.** *Flora of Turkey and the East Aegean Islands*, Vol.**10**, The Edinburgh University Press, Edinburgh, p. 294, 405-406 (1988).
9. **Baytop,T.** *Therapy with medicinal plants in Turkey*, Nobel Tıp Kitabevleri, İstanbul, pp.189 (1999).
10. **Şener, B., Kusmenoğlu, Ş., Mutlugil, A., Bingöl, F. "**A study with seed oil of *N.sativa*" *J. Fac. Pharm. Gazi* **2**, 1-7 (1985).

11. **Akçasu, A., Kavalalı, G.** "The fatty acids of *N.damascena* L. seed oil" *Acta Pharmaceutica Turcica* **32**, 41-43 (1990).

12. **Üstün, G., Kent, L., Çekin, N., Civelekoğlu, H. "**Investigation of the technological properties of *Nigella sativa* (Black Cumin) seed oil" *J. Am. Oil Chem.* **67**, 958-960 (1990).

13. **Nergiz, C., Ötleş, S.,** "Chemical composition of *Nigella sativa L*. Seeds" *Food Chem*., **48**, 259-261 (1993).

14. **Türker, L., Bayrak, A. "**Çörek otu (*Nigella sativa* L.*)*'nun sabit ve uçucu yağ kompozisyonunun araştırılması" *Standard*, **430**, 128-137 (1997).

15. **Meral, I., Yener, Z., Kahraman, T., Mert, N.** "Effect of *Nigella sativa* on glucose concentration, lipid peroxidation, anti-oxidant defence system and liver damage in experimentally-induced diabetic rabbits", *J Vet Med A*, **48,** 593-599 (2001).

16. **Kanter, M., Meral, I., Dede,S., Cemek,M., Ozbek, H., Uygan,I., Gündüz,H.** "Effects of *Nigella sativa* L. and *Urtica dioica* L. on Lipid Peroxidation, antioxidant enzyme systems and some liver enzymes in CCl_4-treated rats" *Journal of Veterinary Medicine A*, **50**, 264-268 (2003).

17. **National Committee for Clinical Laboratory Standards (NCCLS)** Performance Standards for Antimicrobial Disc Susceptibility Tests, Approved Standard, 8.Ed., M2-A8, Wayne, PA (2003).

18. **Awadh Ali, N.A., Jülich, W-D., Kusnick, C., Lindequist, U.** " Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities" *J Ethnopharm*., **74**: 173-179 (2001).

Received: 27.07.2005

Accepted: 28.11.2005