VERSITA

Biologia, Bratislava, **61**/3: 285—288, 2006
Section Cellular and Molecular Biology
DOI: 10.2478/s11756-006-0052-6

In vitro anti-microbial activity of extracts from the callus cultures of some *Nigella* species

Premysl Landa[1], Petr Marsik[2]*, Tomas Vanek[2], Vojtech Rada[3] & Ladislav Kokoska[1]

[1]*Department of Crop Sciences and Agroforestry, Institute of Tropics and Subtropics, Czech University of Agriculture Prague, Czech Republic*
[2]*Department of Plant Tissue Cultures, Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, CZ-16610 Prague 6 – Dejvice, Czech Republic; phone: ++ 420 220183476, fax: ++ 420 220183574, e-mail: marsik@uochb.cas.cz*
[3]*Department of Microbiology, Nutrition and Dietetics, Faculty of Agrobiology, Food and Natural Resources, Czech University of Agriculture Prague, Czech Republic*

Abstract: Crude methanol extracts from callus cultures of *Nigella arvensis*, *N. damascena*, *N. hispanica, N. integrifolia*, and *N. sativa* were investigated for their anti-microbial activity. Growth inhibition was determined in Gram-positive and Gram-negative bacterial strains as well as in yeast by using a broth-microdilution method. The results showed that the extracts of all calli tested exhibited significant anti-microbial activity, especially against *Bacillus cereus*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. Compared with other *Nigella* species, a callus culture of *N. hispanica* was the most effective against the microorganisms used in this study.

Key words: *Nigella*, callus culture, antimicrobial activity.

Introduction

The genus *Nigella* (family *Ranunculaceae*) comprises 14 species of annual herbaceous plants of Mediterranean and West Asian origin, including some species of commercial importance, for example spices, aromatic, medicinal, and ornamental plants (Zohary, 1983).

Nigella sativa L. is the most studied species of the genus. Its seeds were investigated for a wide range of biological activities, e.g. anti-oxidant, anti-inflammatory, immunomodulatory, anti-ulcer, anti-parasitic (Ali & Blunden, 2003), and anti-tumour activities (Musa et al., 2004). A number of reports have been published on the anti-fungal and anti-bacterial actions of *N. sativa* seed extracts or its essential oil (Agarwal et al., 1979; Khan et al., 2003). The seeds contain fixed oil, essential oil, alkaloids, steroids, and phenolic compounds (Akram Khan, 1999). Among a large number of previously isolated compounds, thymohydroquinone (El-Alfy et al., 1975) and thymoquinone (Aljabre et al., 2005) were identified as the main anti-microbial principles of the plant.

Previous studies on seeds of *Nigella damascena* L. revealed analgesic, diuretic (Bekemeier et al., 1967), and estrogenic activities (Agradi et al., 2001). Alkaloids (Dopke & Fritsch, 1970), flavonoids (Lebreton, 1986), and terpenes (Tillequin et al., 1976) have been reported as the major components of this plant.

Little information is available about the chemistry (Kirichenko et al., 1972; Aitzetmuller et al., 1997) and pharmacological action (Atia et al., 2002) of *Nigella arvensis* L. There have been no studies on the phytochemistry and pharmacology of *Nigella integrifolia* Regel and *Nigella hispanica* L.

The past few decades have seen increasing scientific interest in the both growth of plant tissue culture and the commercial development of this technology as means of producing valuable phytochemicals (Havkin-Frenkel et al., 1997). Callus cultures from medicinal plants have been established under suitable conditions to enable production of anti-microbial substances *in vitro* (Chintalwar et al., 2003, Wolters & Eilert, 2003). Recently, papers investigating the anti-microbial activity of extracts from calli of different medicinal plant species have been published (Sokmen et al., 1999, Furmanowa et al., 2002). Although very few plant cell processes are operating commercially, the most successful commercial pharmaceuticals produced from undifferentiated cell cultures are anti-biotic compounds (Khafagi et al., 2003).

The aim of this study was to determine the *in vitro*

* Corresponding author

anti-microbial activity of crude methanol extracts from callus cultures of five *Nigella* species against Gram-positive and Gram-negative bacterial strains as well as against yeast, using the broth-microdilution method.

Material and methods

Plant materials
The seeds of all species tested were obtained from the botanical gardens international exchange service (Index Seminum). The plants were grown on the experimental fields of the Czech University of Agriculture Prague (CUA Prague). The voucher specimens authenticated by Dr. Kokoska were deposited in the Institute of Tropics and Subtropics Agriculture Herbarium, CUA Prague.

Callus culture conditions
The callus tissue was derived from the stems of seedlings. Explants were cultured on solid Murashige and Skoog medium (Murashige & Skoog, 1962) supplemented with 3% of sucrose, naphthalene acetic acid (1 mg/L), and kinetin (0.5 mg/L). The newly initiated calli were then transferred to fresh media containing the same components. Cultures were maintained in permanent darkness for one year at 25 ± 2 °C (except for *N. arvensis* which was under 16h light / 8h dark period). The subculture intervals and harvesting time ranged from 14 to 30 days depending on the particular species.

All callus cultures are included in collection of the Department of Plant Tissue Cultures of the Institute of Organic Chemistry and Biochemistry of the Academy of Sciences of the Czech Republic. The callus culture strain numbers are given in Table 1.

Preparation of extracts
The freshly harvested cell mass from callus cultures was lyophilised and the resulting powdered material of each sample (10 g) was extracted using a Soxhlet apparatus with methanol for 24 h. The extract was subsequently filtered and concentrated *in vacuo* at 40 °C. The residue was resolved in 10% (v/v) solution of dimethylsulfoxide (DMSO) in Tris buffer saline (TBS) of pH 7.6 (Sigma, USA) to create a concentration of 200 mg/mL of stock solution. All samples were sterilized by filtration through a 0.23 μm membrane filter and stored at +4 °C until tested. The yields of dried residues are shown in Table 1.

Microorganisms and media
The following strains of bacteria were used: *Bacillus cereus* ATCC 11778, *Bacillus subtilis* ATCC 6633, *Bacteroides fragilis* ATCC 25285, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Streptococcus pneumoniae* ATCC 6305, and *Streptococcus pyogenes* ATCC 19615. The yeast strain used in this study was *Candida albicans* ATCC 10231.

B. fragilis was grown in Wilkins-Chalgren anaerobe broth under conditions using Anaerobic Jar HP11 (Oxoid, UK). Streptococci were grown in Brain-heart infusion broth and other microorganisms were tested in Mueller-Hinton broth.

All microbial strains and cultivation media were purchased from Oxoid (UK). The susceptibilities of bacteria to ciprofloxacin and yeast to nystatin (Sigma, USA) were checked as positive controls (Table 1).

Anti-microbial test
In vitro anti-microbial activity was determined by the broth-microdilution method (Jorgensen et al., 1999) using 96-well microtitre plates. Two-fold dilutions (six) of each extract tested were carried out, starting from a concentration of 100 mg/mL. Each well was inoculated with 5 μL of bacterial suspension at a density of 10^7 CFU/mL. The microtitre plates were incubated at 37 °C for 24 h (or 48 h for the yeast) and then observed for the minimum inhibitory concentration (MIC), which was defined as the lowest concentration that inhibited any visible growth. A solution of DMSO (5% v/v) in TBS was assayed as the negative control, simultaneously. All samples were tested in triplicate.

Results and discussion

Table 1 gives a summary of the investigated *Nigella* species and the results of anti-microbial screening. It was observed that the extracts from callus cultures of all species showed significant anti-microbial activity, especially against *Bacillus cereus*, *Staphylococcus aureus* and *Staphylococcus epidermidis* (with MICs values ranging from 12.5 to 50 mg/mL). Moderate or weak inhibition activity was observed against *Bacillus subtilis*, *Enterococcus faecalis*, and *Bacteroides fragilis.* No significant activity was seen against *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, or *Candida albicans* (except for the extracts from *N. hispanica* callus).

The extracts from seeds of *N. sativa* (Hanafy & Hatem, 1991) and *N. damascena* (Fico et al., 2004), as well as the extracts from cell cultures of *N. sativa* (Sokmen et al., 1999) have previously exhibited significant anti-microbial activity. In addition, both plants are generally considered as the most pharmacologically active species of the genus (Riaz et al., 1996). However, our results showed that extracts from *N. arvensis*, *N. integrifolia*, and *N. hispanica* calli were more effective than those of *N. sativa* or *N. damascena*, whereas the extract from *N. hispanica* callus culture was more effective than other *Nigella* calli against the microorganisms used in this study. A significant inhibitory activity has also been observed for cell culture extract of *N. arvensis.*

Based on our results, we suppose that higher activity of *N. arvensis*, *N. integrifolia*, and *N. hispanica* extracts then those of *N. sativa* and *N. damascena* could be caused due to production of anti-microbial compounds previously detected in seeds e.g. quinones (Mouhajir et al., 1999) or due to presence of new type of active structures. Since variances in chemical composition of callus tissues and intact plants have been described for number of plant species (Lopez et al., 1999; Taniguchi et al., 2002; Schmeda-Hirschmann et al., 2005), we also suggest that the resulting anti-microbial

Table 1. Minimum inhibitory concentrations of methanol extracts from callus cultures of four *Nigella* species (mg/mL).[a]

Microorganisms	Plant species and cell culture strain/yield of dried residues (g)					Positive and negative controls		
	NA 1AL01 (3.715)	ND 1AD01 (5.180)	NH 1AD01 (1.955)	NI 1AD01 (2.511)	NS 1AD01 (5.308)	CIP (μg/mL)	NS (μg/mL)	DMSO (5%)
Gram-positive								
Bacillus cereus	25	50	25	50	50	1	n.d.	n.a.
Bacillus subtilis	50	n.a.	25	50	50	2	n.d.	n.a.
Enterococcus faecalis	100	n.a.	50	50	50	1	n.d.	n.a.
Staphylococcus aureus	25	50	12.5	50	50	0.5	n.d.	n.a.
Staphylococcus epidermidis	25	50	12.5	50	50	1	n.d.	n.a.
Streptococcus pneumoniae	100	n.a.	25	n.a.	n.a.	1	n.d.	n.a.
Streptococcus pyogenes	n.a.	n.a.	50	n.a.	n.a.	1	n.d.	n.a.
Gram-negative								
Bacteroides fragilis	50	50	25	n.a.	50	2	n.d.	n.a.
Escherichia coli	n.a.	n.a.	50	50	n.a.	0.015	n.d.	n.a.
Pseudomonas aeruginosa	50	n.a.	50	100	n.a.	0.25	n.d.	n.a.
Yeast								
Candida albicans	100	n.a.	50	n.a.	n.a.	n.d.	4	n.a.

[a] NA: *Nigella arvensis*, ND: *Nigella damascena*, NH: *Nigella hispanica*, NI: *Nigella integrifolia*, NS: *Nigella sativa*, CIP: ciprofloxacin, NS: nystatin, DMSO: dimethylsulfoxide, n.a.: not active (>100), n.d.: not determined.

effect of the extracts tested could be significantly affected by possible differences in chemical composition between the seeds and calli.

In summary, the results obtained in the present investigation demonstrate that *N. arvensis* and *N. hispanica* calli can be considered to be more potent sources of anti-microbial compounds then other *Nigella* species investigated. However, further phytochemical studies are required to determine the types of compounds responsible for the anti-bacterial effects of these species. In addition, our results indicate that investigation of *N. arvensis*, *N. integrifolia*, and *N. hispanica* seeds for anti-microbial activity could bring valuable results.

Acknowledgements

This work was supported by the Grant Agency of the Czech Republic (project no. 525/02/0257) and COST project 926.10.

References

Agarwal, R., Kharya. M.D. & Shrivastava, R. 1979. Antimicrobial and antihelmintic activities of the essential oil of *Nigella sativa* Linn. Indian J. Exp. Biol. **17:** 1264–1265.

Agradi, E., Fico, G., Cillo, F., Francisci, C. & Tome, F. 2001. Estrogenic activity of phenolic compounds from *Nigella damascena* evaluated using a recombinant yeast screen. Planta Med. **67:** 553–555.

Aitzetmuller, K., Werner, G. & Ivanov, S.A. 1997. Seed oils of *Nigella* species and of closely related genera. Oil Corps Gras Lipides **4:** 385–388.

Akram Khan, M. 1999. Chemical composition and medicinal properties of *Nigella sativa* Linn. Inflammopharmacology **7:** 15–35.

Ali, B.H. & Blunden, G. 2003. Pharmacological and toxicological properties of *Nigella sativa*. Phytother. Res. **17:** 299–305.

Aljabre, S.H.M., Randhawa, M.A., Akhtar, N., Alakloby, O.M., Alqurashi, A.M. & Aldossary, A. 2005. Antidermatophyte activity of ether extract of *Nigella sativa* and its active principle, thymoquinone. J. Ethnopharmacol. **101:** 116–119.

Atia, F., Mountian, I., Simaels, J., Waelkens, E. & Van Driessche, W. 2002. Stimulatory effects on Na^+ transport in renal epithelia induced by extracts of *Nigella arvensis* are caused by adenosine. J. Exp. Biol. **205:** 3729–3737.

Bekemeier, H., Leuschner, G. & Shmollack, W. 1967. Antipyretische, antioedematose und analgetische wirkung von damascenin im vergleich mit acetylsalicylsaure und phenylbutazon. Arch. Int. Pharmacodyn. **168:** 199–211.

Chintalwar, G.J., Gupta, S., Roja, G. & Bapat, V.A. 2003. Protoberberine alkaloids from callus and cell suspension cultures of Tinospora cordifolia. Pharm. Biol. **41:** 81–86.

Dopke, W. & Fritsch, G. 1970. Alkaloid content of *Nigella damascena*. Pharmazie **25:** 69–70.

El-Alfy, T.S., El-Fatatry, H.M. & Toama, M.A. 1975. Isolation and structure assignment of an antimicrobial principle from the volatile oil of *Nigella sativa* L. seeds. Pharmazie **30:** 109–111.

Fico, G., Panizzi, L., Flamini, G., Braca, A., Morelli, I., Tome, F. & Cioni, P.L. 2004. Biological screening of *Nigella damascena* for antimicrobial and molluscicidal activities. Phytother. Res. **18:** 468–470.

Furmanowa, M., Nosov, A.M., Oreshnikov, A.V., Klushin, A.G., Kotin, M., Starosciak, B., Sliwinska, A., Guzewska, J. & Bloch, R. 2002. Antimicrobial activity of *Polyscias filicifolia* cell biomass extracts. Pharmazie **57:** 424–426.

Hanafy, M.S. & Hatem, M.E. 1991. Studies on the antimicrobial activity of *Nigella sativa* seed (black cumin). J. Ethnopharmacol. **34:** 275–278.

Havkin-Frenkel, D., Dorn, R. & Leustek, T. 1997 Plant tissue culture for production of secondary metabolites. Food Technol. **51:** 56–61.

Jorgensen, J.H., Turnidge, J.D. & Washington, J.A. 1999. Antibacterial susceptibility tests: dilution and disk diffusion methods, pp. 1526–1543. In: Murray, P.R. (ed.) Manual of Clinical Microbiology, ASM Press, Washington, D.C.

Khafagi, I., Dewedar, A. & Amein, M. 2003. Opportunities of finding novel anti-infective agents from plant cell cultures. Curr. Med. Chem. **2:** 191–211.

Khan, M.A.U., Ashfaq, M.K., Zuberi, H.S., Mahmood, M.S. & Gilani, A.H. 2003. The *in vitro* antifungal activity of the aqueous extract from *Nigella sativa* seeds. Phytother. Res. **17:** 183–186.

KIRICHENKO, B.M., KORESHCHUK, K.E. & DROZD, G.A. 1972. Rhamnocitrin – a new component of *Nigella arvensis*. Him. Prir. Soedin. **8:** 672.

LEBRETON, P. 1986. Les flavonoides, marqueurs systematiques chez les Renuncolacees. Plant Med. Phytother. **4:** 275–286.

LOPEZ, M.G., SANCHEZ-MENDOZA, I.R., OCHOA-ALEJO, N. 1999. Compartive study of volatile components and fatty acids of plants and in vitro cultures of parsley (*Petroselinum crispum* (Mill) Nym ex hill). J. Agr. Food Chem. **47:** 3292–3296.

MOUHAJIR, F., PEDERSEN, J.A., REJDALI, M. & TOWERS, G.H.N. 1999. Antimicrobial thymohydroquinones of Moroccan *Nigella sativa* seeds detected by electron spin resonance. Pharm. Biol. **37:** 391–395.

MURASHIGE, T. & SKOOG, F. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant. **15:** 473–498.

MUSA, D., DILSIZ, N., GUMUSHAN, H., ULAKOGLU, G. & BITIREN, M. 2004. Antitumor activity of an ethanol extract of *Nigella sativa* seeds. Biologia, Bratislava **59:** 735–740.

RIAZ, M., MEENA, S. & CHAUDHARY, F.M. 1996. Chemistry of the medicinal plants of the genus *Nigella* (family – *Ranunculaceae*). Hamdard Med. **39:** 40–45.

SCHMEDA-HIRSCHMANN, G., JORDAN, M., GERTH, A., WILKEN, D. 2005. Secondary metabolite content in rhizomes, callus cultures and in vitro regenerated plantlets of *Solidago chilensis*. Z. Naturforsch. **C60:** 5–10.

SOKMEN, A., JONES, B.M. & ERTURK, M. 1999. Antimicrobial activity of extracts from the cell cultures of some Turkish medicinal plants. Phytother. Res. **13:** 355–357.

TANIGUCHI, S., IMAYOSHI, Y., KOBAYASHI, E., TAKAMATSU, Y., ITO, H., HATANO, T., SAKAGAMI, H., TOKUDA, H., NISHINO, H., SUGITA, D., SHIMURA, S., YOSHIDA, T. 2002. Production of bioactive triterpenes by *Eriobotrya japonica* calli. Phytochemistry **59:** 315–323.

TILLEQUIN, F., LECONTE, C. & PARIS, M. 1976. Sesquiterpene hydrocarbons from *Nigella damascena* seeds. Planta Med. **30:** 59–61.

WOLTERS, B. & EILERT, U. 1981. Antimicrobial substances in callus cultures of *Ruta graveolens*. Planta Med. **45:** 166–174.

ZOHARY, M. 1983. The genus *Nigella* (*Ranunculaceae*) – a taxonomic revision. Plant Syst. Evol. **142:** 71–107.

Received September 27, 2005
Accepted January 31, 2006