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Antimitotic and antibacterial effects of the *Nigella sativa* L. Seed

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Abstract — A large number of medicinal plants have therapeutic potentials. *Nigella sativa* L. (Ranunculaceae), known commonly as "black cumin", is a herbaceous plant that grows in Mediterranean countries. Recently, many biological activities of *Nigella sativa* L. seeds have been reported, including: antioxidant, anti-inflammatory, anticancer and antimicrobial. In this study several seed extracts from *Nigella sativa* L. have been tested for antimitotic and antibacterial activity. *Allium cepa* L. has been used for evaluating cytotoxicity. Each extract was toxic on root number and length and reduced the mitotic index. All extracts shows antimitotic activities but ether extract has found the most effective on reducing mitose in root meristem cells. Ether extract has also found effective against Gram-positive bacteria.

Key words: *Allium cepa* L., antibacterial, antimitotic, cytotoxicity, mitotic index, *Nigella sativa* L.

INTRODUCTION

A large number of medicinal plants have therapeutic potentials. *Nigella sativa* L. (Ranunculaceae), known commonly as "black cumin", is a herbaceous plant that grows in Mediterranean countries and is also cultivated in Turkey. Seeds of *Nigella sativa* L. have been employed for thousands years as a spice and food preservative. The oil and seed constituents have shown potential medicinal properties in traditional medicine (Salem 2005).

Recently, many biological activities of *Nigella sativa* L. seeds have been reported, including: antioxidant, anti-inflammatory, anticancer and antimicrobial.

Nigella sativa L. seeds contains a large amount of fixed oils (Kökdil and Yilmaz 2005) and the main constituent of the seed extract is thymoquinone (Aboul-Ela 2002). Several pharmacological effects have been attributed to active principles of *Nigella sativa* L. which includes thymoquinone, thymohydroquinone, dithymoquinone, thymol, carvacrol, nigellicine, nigellimine-x-oxide, nigellidine and alpha-hedrin (Aljabre *et al.* 2005). Immunomodulatory and therapeutic properties of the *Nigella sativa* L. have been reviewed (Salem 2005). *Nigella sativa* L. seed extract inhibits fungal growth in dermatophytes (Aljabre *et al.* 2005). Cytogenetic studies have been furthered on mouse cells; seed extract of *Nigella sativa* L. has shown protective effects (Aboul-Ela 2002). The fixed oil of *Nigella sativa* L. evidenced by high LD_{50} values in mice and rats (Zaoui *et al.* 2002). Another effect of *Nigella sativa* L. oil is, inducing the oxidative injury in mice (Ilhan *et al.* 2005). *Nigella sativa* L. shows also cytotoxic effects (Swamy and Tan 2000; Thabrew *et al.* 2005). Aquaeous extracts of this plant has anti-inflammatory and analgesic effects on rats and mice (Al-Ghamdi 2001).

The biological effects of "black cumin" is evident. The aim of this study is to determine antimitotic and antibacterial effects of several seed extracts from *Nigella sativa* L.

MATERIAL AND METHOD

Extraction of seeds - Ether extract: 10 g of seeds were placed to a soxhalet and extracted with diethylether at 35 ^{0}C. Ether was evoparated after extraction by a rotary evaporator connected to a vacuum pump.

Ethanolic extract - The plant residue in soxhalet cartridge has been treated with ethanol in a shaker at room temperature. After extraction ethanol was evaporated.

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Preparation of decoction - 10 g of seeds boiled in 200 ml distilled water for 1 h. The extract was than filtered and freeze-dried. After freezing the water was removed by liofilization.

Antimitotic activity - Allium cepa has been used for evaluating cytotoxicity since the early 1920's (GRANT 1982). This method is an easy and sensitive tool for measuring the total toxicity caused by chemical treatments as expressed by growth inhibition of the roots of onion bulbs. It has been reported that the results from Allium test fit in well in a test battery composed of procaryotes and /or other eucaryotes (FISKESJÖ 1993). Small onion bulbs are carefully unscaled and cultivated on top of test tubes filled with the seed extracts. Tap water was used as a control, and for dilution of the seed extracts. The test tubes were kept in an incubator at 24 ± 2 ^{0}C and the test samples were changed daily. After 72 h the roots were counted and their lengths were measured for each onion. When the newly emerged roots measured 2.0 – 3.0 cm, they were fixed. The fixative was glacial acetic acid/absolute alcohol (1/3 v/v). The root tips were kept in the aceto-alcohol solution for 24 h. After fixation, the slides were prepared for examination or the roots were transferred to %70 ethyl-alcohol and stored in a refrigerator. For examination, the root tips were put into a watch glass to which 9 drops of aceto-orcein and 1 drop of 1 M HCl were added and warmed over a flame of spirit lamp for 2-3 min. These were kept at room temperature for 15-30 min. After removing the root caps from well-stained root tips, 1 mm of the mitotic zones were immersed in a drop of %45 acetic-acid on a clean slide and squashed under a cover glass. In order to spread the cells evenly on the surface of the slide, squashing was accomplished with a bouncing action by striking the cover glass with a match stick. MI were expressed in terms of divided cells/ total cells. A statistical analysis was performed on the collected data. The means of the control and seed extracts were obtained from descriptive analysis and an Independet-samples test was performed to obtain P values.

Antibacterial activity - Antibacterial activity was determined by the well diffusion method. Muller Hinton agar plates were seeded with a 24 h culture of the bacterial strains. The inoculum size was adjusted to 0.5 MacFarland turbidity standard (10^8 cfu/ml). Muller-Hinton Agar plates were inoculated with each of these bacterial suspensions using sterile swabs. The dried plant extracts were dissolved in dimethylsulfoxide (DMSO) to give a concentration of 300 mg/ml. Wells were cut into the agar and filled with 75 µl of the plant extracts. Inoculated plates were incubated at 37 °C and 30 °C for *Staphylococcus aureus* ATCC 25923*, Escherichia coli* ATCC 35218*, Enterococcus faecalis* ATCC 51299*, Proteus* sp. and for *Micrococcus luteus* ATCC 9341 respectively.

The antibacterial activity was evaluated by measuring the diameter of inhibition zone. The experiment was carried out in duplicate and the mean of the diameter of the inhibition zones was calculated.

RESULTS AND DISCUSSION

The root length and number for control and for each extract are given in table 1. *Nigella sativa* L. extracts reduced significantly root number and root length when compared with control.

Table 1 — The average root lengths and numbers in control and in extracts after 72 h.

Extract	Average root numbers (±SD)	Average root lengths (mm) (±SD)
Control	27.5 (±4)	29.3 (±3.7)
Ether	2.4 (±3.1)*	0.9 (±1.1)*
Ethanol	13.5 (±9)*	6.2 (±3.6)*
Decoction	12 (±3.3)*	5.7 (±2.2)*

* Significant at 0.05 level

Ether extract was more effective on root length and number when compared with the other extracts.

This results shows that the extracts from *Nigella sativa* L. seeds haves inhibitory effects on root growth and length in *Allium cepa.* In conformity with animal and human cell cytotoxicity (SWAMY and TAN 2000; THABREW *et al.* 2005) it was found that *Nigella sativa* L. seed extracts haves cytotoxic properties also in plant test systems.

In table 2 the mitotic indexes are given for control and for each extract. It is evident that all extracts reduced the mitotic index significantly. The reduction in number of dividing cells in the root meristem shows the antimitotic effects of the substances that found in seed extracts.

Ether extract was more effective on mitotic index when compared with the other extracts. In respect of this results, *Nigella sativa* L. seeds contains antimitotic constituents that can stop the mitosis in anywhere of the cell cycle. Furthermore these constituents probably affects the cytoskeleton or tubulin polymerization or degradation.

Table 2 — The dividing and total cells that counted in microscopic observations and mitotic index (MI) in control and in extracts.

Extract	Total cells	Dividing cells	MI (±SD)
Control	14178	2293	%16 (± 1,6)
Ether	8572	118	%1,4 (± 0,4)*
Ethanol	14222	257	%2 (± 0,6)*
Decoction	19993	305	%1,5 (± 0,7)*

* Significant at 0.05 level

Antibacterial activity - Antibacterial activity of two different extracts of *Nigella sativa* L. has been evaluated in vitro against five bacterial test species, which are known to cause some infections in humans. Among the tested extracts, the most effective one was found to be ether extract (Table 3). The biggest inhibition zone was observed with ether fraction and it inhibited the growth of two Gram-positive bacteria.

Table 3 — Diameters of inhibition zones.

Extract	Zone of inhibiton (mm)				
	S. aureus	E. coli	E. faecalis	Proteus sp.	M. luteus
Ether	15	-	-	-	12
Ethanol	-	-	-	-	-

None of the extracts did show inhibitory effect against *E.coli, Proteus sp.* and *E.faecalis*. It is generally expected that, when antimicrobial activity is measured most of the materials tested would be active against Gram-positive than Gram-negative bacteria (McCUTCHEON *et. al.* 1992). In this study extracts inhibited especially Gram-positive bacteria.

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