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Anti-tuberculosis Activity of *Daucus littoralis* Sibth. et Sm. (Apiaceae) From Turkey[†]

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Abstract

In the course of screening for anti-mycobacterial activity, the authors detected significant activity in the essential oil of *Daucus littoralis* Sibth. et Sm. Composition of the oil was characterized by gas chromatographic (GC) and gas chromatographic/mass spectrometric (GC/MS) analyses. The genus *Daucus* (Apiaceae) is represented in Turkey by six species, one being endemic, *D. conchiteae* W. Greuter. *Daucus carota* L. is a well-known species whose roots are used as food and whose fruit oil is used in perfumery (7,8). Air-dried aerial parts of *D. littoralis* were subjected to water distillation using a Clevenger-type apparatus. The essential oil yield was 0.2% on dry weight basis. The main compound in the oil was found as *cis*-chrysanthenyl acetate (46.8%). The aim of this study was to determine new potent anti-mycobacterial compounds for the treatment of tuberculosis.

Key Word Index

Daucus littoralis, anti-tuberculosis, *Mycobacterium tuberculosis.*

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Introduction

Mycobacteria have recently increased their virulence and tuberculosis (TB) is the most lethal infection worldwide. Thirty million people are expected to catch tuberculosis in the near future by World Health Organization (WHO) (1); one million children per year die from this disease (2,3).

Screening higher plant extracts to isolate novel anti-tubercular natural products and determining their structures continues all over the world. In this study, as part of a search for anti-mycobacterial compounds from the higher plant *Daucus littoralis*, the authors screened the essential oil of the plant against *Mycobacterium tuberculosis*.

The disease appears to be very prevalent particularly among the immune suppressed patients (HIV = Human Immunodeficiency Virus) (4). The main problem in the world is the difficulty of the treatment of tuberculosis disease. *Mycobacteria* are known to be more resistant to many antibiotics, disinfectants and chemicals (4). Plant-based natural compounds like flavones, coumarins, chromones, chalcones, terpenoids, saponins, steroids, phenols, polyphenols and peptides are reported to have anti-mycobacterial activity (5,6). Recently, the inhibitory effects of plant extracts using different parts of plants such as bark, stem, root, leaves and fruits against bacteria and fungi have been investigated (7,8).

Species of the parsley family (Apiaceae) are well-known with regard to their economic importance and diversity of essential oils (9,10). This family is well represented in the Turkey flora with 97 genera and at least 400 species (11).

The genus *Daucus* L. appears to have its center of dispersion in the Mediterranean Region, particularly in North Africa, where strong speciation has taken place. Apart from *D. carota* L., the common carrot, which is cultivated throughout the world, there is one Australian species, naturalized also in Europe, and three other species in the American continent. The rest, as previously stated, are Mediterranean.

The genus *Daucus* (Apiaceae) is represented in Turkey by six species, one of these being endemic. *Daucus littoralis* Sibth. et Sm. is setose, tuberculate or glabrous, annual, with stems up to 40 cm and leaves 2-pinnate; the ultimate segments are narrowly cuneate.

There are only a few phytochemical and biological activity studies on some *Daucus* species. Essential oil studies on *Daucus* species are quite scarce (12,13). To the best of the authors' knowledge, there is no report on the chemistry of the *Daucus* species, which is the subject of this study.

Daucus species have been reported to contain acetone, asarone, choline, ethanol, formic acid, HCN, isobutyric acid, limonene, malic acid, maltose, oxalic acid, palmitic acid, pyrrolidine and quinic acid (12). *Daucus carota* L. is native in Europe and is used as an antibacterial (13,14), anti-oxidant (15,16), stimulant (17), antiseptic, diuretic, hepatoprotective, anti-inflammatory (18,19), anthelmintic, carminative (20), deobstruent, diuretic, galactogogue, ophthalmic and stimulant (21). An infusion is used in the treatment of various complaints including digestive disorders, kidney and bladder diseases and in the treatment of dropsy (22). An infusion of the leaves has been used to counter cystitis and kidney stone formation and to diminish stones that have already formed (23). Carrot leaves contain significant amounts of porphyrins, which stimulate the pituitary gland and lead to the release of increased levels of sex hormones (23).

A warm water infusion of the flowers has been used in the treatment of diabetes (24). The grated raw root, especially of the cultivated forms, is used as a remedy for threadworms.

Table I. Percentage composition of the oil of *Daucus littoralis*

RRI	Compound	%
1032	α-pinene	1.5
1035	α-thujene	tr
1076	camphene	tr
1100	undecane	0.3
1118	β-pinene	tr
1132	sabinene	tr
1174	**myrcene**	**18.7**
1203	limonene	1.7
1218	β-phellandrene	0.2
1244	amylfuran (2-pentyl furan)	tr
1246	(Z)-β-ocimene	1.8
1255	γ-terpinene	0.1
1266	(E)-β-ocimene	0.1
1280	p-cymene	0.3
1290	terpinolene	tr
1296	octanal	tr
1400	nonanal	tr
1429	perillene	0.3
1497	α-copaene	tr
1506	decanal	0.9
1535	β-bourbonene	0.3
1582	***cis*-chrysanthenyl acetate**	**46.8**
1590	bornyl acetate	0.4
1602	β-copaene	tr
1623	β-caryophyllene	1.2
1661	safranal	0.2
1668	(Z)-β-farnesene	4.0
1722	dodecanal	4.4
1744	α-selinene	0.2
1764	*cis*-chrysanthenol	5.6
1766	decanol	0.9
1916	(Z)-7-dodecenol*	tr
1933	tetradecanal	tr
1950	dendrolasin	tr
1958	(E)-β-ionone	tr
1969	(Z)-jasmone	tr
1973	dodecanol	0.9
1984	(Z)-9-tetradecenal*	0.7
2001	isocaryophyllene oxide	0.1
2008	caryophyllene oxide	0.5
2021	8-dodecenol*	0.2
2050	(E)-nerolidol	0.2
2060	9-dodecenol	tr
2131	hexahydrofarnesyl acetone	0.4
2179	tetradecanol	tr
2256	cadalene	tr
2300	tricosane	tr
2500	pentacosane	tr
2622	phytol	0.8
2700	heptacosane	0.7
2842	methyl behenate (methyl docosanoate)	0.5
2900	nonacosane	1.0

RRI = Relative retention indices; tr = trace (< 0.1%); * tentative.

Table II. Susceptibility Test Results obtained by MGIT fluorometric manual method

Species	Final Concentration in MGIT Tubes (µg/mL)				Streptomycin (µg/mL)
	392	196	98	49	1.0
D. littoralis	S	S	R	R	S

S = sensitive; R = resistant.

The root is also used to encourage delayed menstruation. The root of the wild plant can induce uterine contractions and so should not be used by pregnant women (35).

A tea made from the roots is diuretic and has been used in the treatment of urinary stones (18). An infusion is used in the treatment of oedema, flatulent indigestion, and menstrual problems (22). The seed is a traditional "morning after" contraceptive. There is some evidence to uphold this belief, but it requires further investigation (18). Carrot seeds can be abortifacient and so should not be used by pregnant women (23).

This species, which has a very narrow distribution in Turkey with records only from two localities, was recorded from a new locality for the B1 grid square of the Flora of Turkey. In this paper, the authors report their findings on the bioactive components of *D. littoralis* for anti-mycobacterial activity against *Mycobacterium tuberculosis*.

Experimental

Plant material: *Daucus littoralis* was collected from Balikesir in July 2005. A voucher specimen FS 1702 has been deposited at the Herbarium of Department of Biology, Faculty of Science and Letters of the Balikesir University, Balikesir, Turkey. Collection and identification of the plant material were performed by G. Tumen.

Used microorganism: The oil was tested against the reference strain *Mycobacterium tuberculosis* H37Ra (ATCC 25177) in duplicate. Inoculum was prepared by 3–5 days old culture of *M. tuberculosis* by diluting 1:5 from MGIT broth which showed positive.

***Isolation of essential oil*:** Air-dried parts (55 g) were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus to produce the oil (oil yield 0.2%).

GC and GC/MS conditions: *GC/MS:* The GC/MS analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60 m × 0.25 mm, 0.25 µm film thickness) was used with He as carrier gas (0.8 mL/min). GC oven temperature was kept at 60°C for 10 min, programmed to 220°C at a rate of 4°C/min, kept constant at 220°C for 10 min, and then programmed to 240°C at a rate of 1°C/min. Split ratio was adjusted 40:1. The injector temperature was at 250°C. MS were taken at 70 eV. Mass range was from *m/z* 35–450.

GC: The GC analysis was carried out using an Agilent 6890N GC system. In order to obtain the same elution order with GC/MS, simultaneous injection was done by using the same column and appropriate operational conditions. FID temperature was 300°C.

The components of essential oils were identified by comparison of their mass spectra with those in the Baser Library of Essential Oil Constituents, Wiley GC/MS Library, Adams Library, MassFinder Library and were confirmed by comparison of their retention indices. Alkanes were used as reference points in the calculation of relative retention indices (RRI). Relative percentage amounts of the separated compounds were calculated from FID chromatograms. The results of analysis are shown in Table I.

Anti-mycobacterial activity: The *Mycobacteria* Growth Indicator Tube (MGIT) containing 4 mL of modified Middlebrook 7H9 Broth Base was used. Assay was done according to the instruction of the MGIT manual Fluorometric susceptibility test procedure recommended by the manufacturer (Becton Dickinson). OADC enrichment (0.5 mL), a mixture of oleic acid, albumin, dextrose and catalase, was added to each tube. Oil was added in a volume of 0.1 mL to an MGIT. An aliquot (500 µL) of bacterial suspension was dispersed in the tubes. The final concentrations of the oil were 196, 98, 49 and 24 µg/mL. An uninoculated MGIT tube was used as a negative control. The control tube contained organisms only and not the oil. Blood Agar was used for checking the growth of other bacteria. The vials were incubated at 37°C and MIC was determined to be the lowest dilution which read negative by MicroMGIT Fluorescence reader within two days of when the controls turned positive. Tubes were read daily starting on the second day of incubation using MicroMGIT Flourescence reader with a long wave UV light.

Results and Discussion

The oil was isolated from the aerial parts of *Daucus littoralis* Sibth. & Sm. using a Clevenger-type apparatus. Oil was analyzed by GC and GC/MS. The composition of essential oils is shown in Table I. Fifty-two compounds were characterized making up of 95.9% of the oil with *cis*-chrysanthenyl acetate (46.8%) and myrcene (18.7%) as main constituents.

The genus *Daucus* (Apiaceae) is represented in Turkey by six species, one being endemic, *D. conchiteae* W.Greuter. *Daucus carota* L. is a well-known species whose roots are used as food and whose fruit oil is used in perfumery (26,27). This is the first report on the chemistry and anti-mycobacterial activity of *D. littoralis*. In vitro evaluation of anti-mycobacterial activity was carried out using the MGIT *Mycobacteria* Growth Indicator Tube, 7H9 Broth Base (Becton Dickinson).

Daucus littoralis oil and the reference drug, Streptomycin, were assayed against *M. tuberculosis* ATCC 25177 (H37Ra). *Daucus littoralis* showed the best activity in the value of MIC 196 µg/mL against the microorganism. *Mycobacterium tuberculosis* was sensitive to standard drug, Streptomycin, in the value of 1.0 µg/mL (Table II).

Myrcene and limonene were main components of the hydrocarbons present in the oil. Decanal, dodecanal and

cis-chrysanthenyl acetate were the major oxygen-containing constituents (25). The oil and the main oxygen-containing aliphatic components showed a remarkable anti-mycobacterial activity against *Mycobacterium tuberculosis*.

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