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Antimycobacterial Activity of Some Turkish Plants

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Abstract

In this study, 70% ethanol extracts from 98 different plant species distributed among 21 families were evaluated for antimycobacterial activity. Sempervirine was isolated from *Buxus sempervirens* with a minimum inhibitory concentration (MIC) of 6.25 µg/ml.

Keywords: Antimycobacterial activity, *Buxus sempervirens*, *Mycobacterium tuberculosis*.

Introduction

Tuberculosis remains a major world health problem, in particular, since the incidence of multidrug-resistant tuberculosis has increased in many countries (Cantrell et al., 1998). In recent years, a large number of reports on the structurally effective new antituberculosis natural products from plants have appeared. In this study, 98 different plant species from Turkey were evaluated for antimycobacterial activity and sempervirine was isolated from *Buxus sempervirens* as an active compound.

Materials and methods

General experimental procedures

¹H NMR and ¹³C NMR spectra were recorded on a Bruker 400 MHz spectrometer. Mass spectra were obtained on a HP-1050 VG-Platform Micromass (EI+) instrument. IR spectra were run in KBr discs on a Perkin Elmer 1330 Spectrophotometer. UV spectra were recorded on a Beckman DU 650 UV-vis Spectrophotometer cabled to a Star LC-20 printer recorder.

Plant materials

Collecting regions and used parts of examined plants are given in Table 1. All voucher specimens were deposited at the “Gazi Üniversitesi Fen Fakültesi Herbaryumu-GAZİ”, in Ankara, Turkey. Plants were identified by M. Vural.

Preparation of extracts and fractions

Ethanol extracts of the air-dried and powdered plant material were prepared by repeated extraction with 70% ethanol at room temperature. For the fractions, air-dried and powdered plant material was sequentially extracted at room temperature with petroleum ether, chloroform, methanol and water. The solvents were removed under vacuum.

Antimycobacterial assay

Antimycobacterial bioassay was performed using the microplate Alamar blue assay (MABA) (Collins & Franzblau 1997). Suspensions of *Mycobacterium tuberculosis* H₃₇Ra strains were prepared at a concentration of about 10⁵ cells/ml, 100 µl of the bacterial suspension were added to each well of a microtiter plate together with the plant extracts in Middlebrook 7H9 medium to the final volume of 200 µl, and the final concentration of the plant extracts were 50, 100 and 200 µg/ml. After incubation for about 7 days, 20 µl of Alamar blue dye were added to the control well. If the dye turned pink, indicating bacterial growth, the dye was then added to all remaining wells in the plate. The results were read in the following day. If the ethanol extracts of plants were active at 200 µg/ml, the minimum inhibitory concentration (MIC) of the fractions of these active plants was determined. For standard tests, the MIC values of rifampin, isoniazid and

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Table 1. Plant materials used for antimycobacterial activity screening and MIC values of their ethanol extracts against *Mycobacterium tuberculosis* H₃₇Ra.

Plants	Locations	Plant part*	MIC (µg/ml)
Amaryllidaceae			
<i>Galanthus elwesii</i> Hooker fil.	Antalya-Akseki	B	200
<i>G. ikariae</i> Baker	Trabzon-Sürmene	B	200
<i>Leucojum aestivum</i> L.	Samsun-Terme	B	100
<i>Narcissus tazetta</i> L. ssp. <i>tazetta</i>	Antalya-Kumluca	B	100
<i>Pancratium maritimum</i> L.	Bartın-İnkum	B	100
Anacardiaceae			
<i>Pistacia atlantica</i> Desf.	Karabük-Safranbolu	Br	–
		L	–
		Fr	–
<i>P. lentiscus</i> L.	Muğla-Bodrum	Br	–
		L	–
<i>P. terebinthus</i> L. ssp. <i>palaestina</i> (Boiss.) Engler	Mersin-Cennet-Cehennem	Br	200
		L	200
Aspleniaceae			
<i>Asplenium septentrionale</i> (L.) Hoffm.	Trabzon-Altındere	A	–
Apiaceae (Umbelliferae)			
<i>Angelica sylvestris</i> L. var. <i>sylvestris</i>	Giresun-Şebinkarahisar	A	–
<i>Apium graveolens</i> L.	Cultivated	L	–
<i>Artemisia squamata</i> L.	Karabük-Safranbolu	A	200
<i>Astrantia maxima</i> Pallas ssp. <i>maxima</i>	Trabzon-Zigana pass	A	–
<i>Coriandrum sativum</i> L.	Cultivated	Fr	50
<i>Foeniculum vulgare</i> Miller	Cultivated	Fr	50
<i>Heracleum platytaenium</i> Boiss.	Trabzon-Hamsiköy	A	–
		Fr	–
<i>Ligusticum alatum</i> (Bieb.) Sprengel	Giresun-Şebinkarahisar	A	–
<i>Pimpinella affinis</i> Ledeb.	Trabzon-Altındere	A	50
<i>P. anisum</i> L.	Cultivated	Fr	200
<i>Smyrniolus olusatrum</i> L.	İstanbul-Baltalimanı	A	–
<i>Tordylium apulum</i> L.	İstanbul-Sarıyer	A	–
Buxaceae			
<i>Buxus sempervirens</i> L.	Rize-Çamlıhemşin	L	50
		Br	100
Campanulaceae			
<i>Campanula alliarifolia</i> Willd.	Giresun-Şebinkarahisar	A	–
<i>C. rapunculoides</i> L. ssp. <i>rapunculoides</i>	Trabzon-Altındere	A	200
<i>C. lactiflora</i> Bieb	Giresun-Şebinkarahisar	A	200
Capparaceae			
<i>Capparis spinosa</i> L. var. <i>spinosa</i>	Muğla-Bodrum	Fr	200
		A	–
Caprifoliaceae			
<i>Lonicera etrusca</i> Santi var. <i>etrusca</i>	Aydın-Kuşadası	A	–
<i>Sambucus ebulus</i> L.	Trabzon-Tonya	A	50
<i>S. nigra</i> L.	İzmir-Bozdağ	A	200
Corylaceae			
<i>Carpinus orientalis</i> Miller ssp. <i>orientalis</i>	Trabzon-Altındere	L	–
<i>Corylus avellana</i> L. var. <i>avellana</i>	Giresun-Şebinkarahisar	L	–
Cucurbitaceae			
<i>Ecballium elaterium</i> (L.) A. Rich.	Muğla-Bodrum	A	–
		Fr	–
<i>Momordica charantia</i> L.	Bursa-Akköy	Fr	200
Ericaceae			
<i>Erica arborea</i> L.	İstanbul-Büyükdada	A	–
<i>Rhododendron luteum</i> Sweet	Trabzon-Altındere	A	–
<i>R. ponticum</i> L. ssp. <i>ponticum</i>	Trabzon-Tonya	A	–
<i>Vaccinium arctostaphylos</i> L.	Trabzon-Tonya	A	–

Table 1. Continued

Plants	Locations	Plant part*	MIC (µg/ml)
Euphorbiaceae			
<i>Euphorbia helioscopia</i> L.	Nevşehir-Ürgüp	A	–
<i>E. macroclada</i> Boiss.	Ankara-Çankaya	A	–
<i>E. oblongifolia</i> (C. Koch) C. Koch	Trabzon-Zigana pass	A	–
Fagaceae			
<i>Quercus ithaburensis</i> ssp. <i>macrolepis</i>	Ankara-Çankaya	L	–
Gentianaceae			
<i>Gentiana cruciata</i> L.	Trabzon-Zigana pass	A	200
<i>G. septemfida</i> Pallas	Trabzon-Zigana pass	A	–
Leguminosae			
<i>Acacia dealbata</i> Link	Cultivated	L+F1	200
<i>A. cyanophylla</i> Lindley	Cultivated	L	50
<i>Albizia julibrissin</i> Durazz.	Cultivated	L+F1	–
<i>Astragalus caraganae</i> Fisch et Mey.	Van-Akdamar	A	–
<i>Astragalus pycnocephalus</i> Fischer var. <i>pycnocephalus</i>	Van-Akdamar	A	–
<i>Caesalpinia gillesii</i> Wall. ex Hook.	Cultivated	A	200
<i>Calicotome villosa</i> (Poiret) Link	İstanbul-Büyükdada	A	100
<i>Cicer arietinum</i> L.	Cultivated	A	–
<i>Coronilla varia</i> L. ssp. <i>varia</i>	Van-Campus of the Univ.	A	200
<i>Galega officinalis</i> L.	Bolu-Bolu mountain	A	–
<i>Genista sessilifolia</i> DC.	Ankara-Lalahan	A	–
<i>Genista albida</i> Willd.	Ankara-Beynam	A	–
<i>Gonocytisus pterocladus</i> (Boiss.) Spach	Hatay-Narlıca	A	–
<i>Gonocytisus angulatus</i> (L.) Spach	İzmir-Bornova	A	–
<i>Lathyrus pratensis</i> L.	Giresun-Şebinkarahisar	A	–
<i>Lotus corniculatus</i> L. var. <i>corniculatus</i>	Van-Muradiye	A	–
<i>Lupinus angustifolius</i> L.	Aydın-Avcılar	A	–
<i>Mellilotus officinalis</i> (L.) Desr.	Ankara-Batıkent	A	–
<i>Parkinsonia aculeata</i> L.	Cultivated	L	200
<i>Robinia pseudoacacia</i> L.	Cultivated	L+F1	–
<i>Spartium junceum</i> L.	İstanbul-Kartal	A	50
<i>Vicia faba</i> L.	Cultivated	A	–
<i>V. sativa</i> L.	İstanbul-Tellibaba	A	200
<i>Wisteria sinensis</i> Sweet	Cultivated	L+F1	–
Liliaceae			
<i>Allium scorodoprasum</i> L. subsp. <i>rotundum</i> (L.) Stearn	Van-Akdamar	B	–
		F1	–
<i>Asphodelus aestivus</i> Brot.	Antakya-Arsuz	L	–
<i>Lilium candidum</i> L.	Antalya-Aspendos	B	–
<i>Ornithogalum armeniacum</i> Baker	Aydın-Avcılar	F1	–
<i>O. umbellatum</i> L.	Afyon-Dinar	B	–
		F1	100
<i>Urginea maritima</i> (L.) Baker	İzmir-Kesre	B	–
Myrtaceae			
<i>Myrtus communis</i> L.	İzmir-Aliaga	L	–
Ranunculaceae			
<i>Adonis flammea</i> Jacq.	Ankara-Batıkent	A	–
<i>Consolida orientalis</i> (Gay) Schröd.	Van-Campus of the Univ.	A	–
<i>Ranunculus constantinopolitanus</i> (DC.) d'Urv.	Bolu-Gerede	A	–
<i>R. illyricus</i> L.	Van-Muradiye	A	–
<i>Thalictrum minus</i> L. var. <i>majus</i> (Cranta) Crepin	Giresun-Şebinkarahisar	A	200
Rosaceae			
<i>Alchemilla mollis</i> (Buser) Rothm.	Trabzon-Zigana pass	A	–
<i>Armeniaca vulgaris</i> Lam.	Cultivated	L	–
		P	–
		S	–

Table 1. Continued

Plants	Locations	Plant part*	MIC (µg/ml)
<i>Cerasus avium</i> (L.) Moench	Cultivated	L	50
<i>C. vulgaris</i> Miller	Cultivated	L	–
<i>Chanomeles speciosa</i> (Sweet) Nakai	Cultivated	L+Fr	50
<i>Cydonia oblonga</i> Miller	Cultivated	L	–
<i>Fragaria vesca</i> L.	Trabzon-Zigana pass	L+Fr	–
<i>Laurocerasus officinalis</i> Roemer	Giresun-Giresun castle	L	–
		Fr	–
<i>Malus sylvestris</i> Miller ssp. <i>mitis</i> (Wallr.) Mansf.	Cultivated	L	–
<i>Persica vulgaris</i> Miller	Cultivated	L	–
<i>Potentilla recta</i> L.	Van-Muradiye	A	–
<i>Pyracantha coccinea</i> Roemer	Muğla-Bodrum	A	–
<i>Pyrus communis</i> L. ssp. <i>sativa</i> (DC.) Hegi	Kültür	L	–
<i>Rosa canina</i> L.	Trabzon-Akçaabat	L+Fr	–
<i>Rubus sanctus</i> Schreber	Trabzon-Maçka	L+Fr	–
Scrophulariaceae			
<i>Melampyrum arvense</i> L. var. <i>elatius</i> Boiss.	Trabzon-Tonya	A	–
<i>Verbascum vulcanicum</i> Boiss. et Heldr.	Nevşehir-Hacıbektaş	A	–
<i>Veronica anagallis-aquatica</i> L.	Giresun-Şebinkarahisar	A	–
Ulmaceae			
<i>Celtis glabrata</i> Steven et Planchon	Kastamonu-Araç	L	–

* A, aerial; B, bulbs; Br, branches; Fl, flowers; Fr, fruits; L, leaves; P, pulp; S, seeds; –, inactive.

kanamycin were determined each time. The acceptable MIC ranges of drugs were 0.0047–0.0095, 0.05–0.1 and 2.5–5.0 µg/ml, respectively.

Isolation of sempervirine

(+)-Sempervirine, a major alkaloid, was isolated from the alkaloid fraction of the air-dried leaves of *Buxus sempervirens* as previously reported, and its physical and spectral data were in agreement with those published in the literature (Atta-ur-Rahman et al., 1991).

Results and Discussion

The extracts and fractions that could not prevent growth of the microorganism up to a concentration of 200 µg/ml were considered inactive. Eighteen of the 98 plants inhibited growth at a concentration of 200 µg/ml, 6 plants inhibited growth at 100 µg/ml, and nine plants inhibited growth at 50 µg/ml concentrations, as listed in Table 1. Fractions prepared from nine active plants at the concentration 50 µg/ml were tested using the same method. As shown in Table 2, the highest level of antimycobacterial activity was found in the chloroform fraction of *B. sempervirens* and petroleum ether fraction of *Pimpinella affinis*. An alkaloid extract obtained from the leaves and stems of *B. sempervirens* was previously reported for use in the treatment of tuberculosis, although its exact potency was not reported (Merck and Co., 1957). TLC analysis of the fractions of *B. sempervirens* showed that alkaloids were the major compound in chloroform and methanol

Table 2. Minimum inhibitory concentrations of fractions against *Mycobacterium tuberculosis* H₃₇Ra.

Plants	MIC (µg/ml)			
	P	C	M	W
<i>Acacia cyanophylla</i>	100	100	50	50
<i>Buxus sempervirens</i>	200	25	50	200
<i>Cerasus avium</i>	50	50	50	–
<i>Chanomeles speciosa</i>	100	50	–	–
<i>Coriandrum sativum</i>	50	50	–	–
<i>Foeniculum vulgare</i>	100	50	200	–
<i>Pimpinella affinis</i>	25	100	–	200
<i>Sambucus ebulus</i>	100	50	100	–
<i>Spartium junceum</i>	200	50	–	–

P, petroleum ether fraction; C, chloroform fraction; M, methanol fraction; W, water fraction.

fractions. On the basis of these results, the alkaloid fraction obtained from the leaves of *B. sempervirens* and sempervirine isolated from the alkaloid fraction were tested against *M. tuberculosis* separately. The MIC values were determined as 12.5 and 6.25 µg/ml, respectively. Further investigation of other plants with high activity is currently in progress.

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