Turk J. Pharm. Sci. 7 (2), 139-150, 2010

Original article

TRADITIONAL MEDICINE IN BİLECİK PROVINCE (TURKEY) AND ANTIMICROBIAL ACTIVITIES OF SELECTED SPECIES

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Abstract

Traditional medicine in North-West of Turkey (Bilecik province) was studied during a three-month field study by interviewing local informants from several districts. 64 plant samples belonging to 33 families were recorded to be used in the treatment of 26 different diseases mainly for gastrointestinal disorders (18%), respiratory disorders (11%), rheumatism (15%) and skin disorders (8%). Uses of Cionura erecta, Echium plantagineum, Neslia apiculata, Stachys thirkei, Oxalis articulata, Consolida regalis subsp. paniculata and Solanum alatum in traditional medicine have been reported for the first time herein. The plant species used to treat infections and skin disorders were tested for antimicrobial activity. Petroleum ether and ethanol extracts were prepared from the aerial parts of Hedera helix, Lavandula stoechas subsp. cariensis, Plantago major, Teucrium chamaedrys subsp. chamaedrys and Teucrium polium and tested in vitro against 7 bacterial and one fungal strains using microbroth dilution technique according to Clinical and Laboratory Standards Institute (CLSI). The results indicate that the ethanolic extracts of L. stoechas subsp. cariensis and P. major have shown the strongest activity against Staphylococcus aureus with a MIC value of 19.52 µg/ml. The petroleum ether extracts of T. polium and T. chamaedrys subsp. chamaedrys have exhibited moderate activity against Klebsiella pneumoniae (MIC: 156.2 µg/ml). None of the extracts tested have shown antifungal activity against C. albicans.

Key words: *Traditional medicines, Bilecik (Turkey), Antimicrobial activity, Plant.*

Bilecik İlinde Geleneksel Tıp ve Seçilmiş Türlerin Antimikrobiyal Aktivitesi

Kuzeybatı Türkiye (Bilecik ili) geleneksel ilaçları, 3 aylık bir arazi çalışması süresince çeşitli ilçelerden yerel bilgi veren kimseler ile görüşülerek çalışılmıştır. 33 familyaya ait 64 bitki örneğinin başlıca gastro-intestinal sistem rahatsızlıklar (18%), solunum sistemi rahatsızlıkları (11%), romatizma (15%) ve deri hastalıkları (8%) olmak üzere 26 farklı hastalığın tedavisinde kullanıldığı kaydedilmiştir. Cionura erecta, Echium plantagineum, Neslia apiculata, Stachys thirkei, Oxalis articulata, Consolida regalis subsp. paniculata ve Solanum alatum'un geleneksel ilaç olarak tıbbi kullanılışları ilk kez bildirilmiştir. Enfeksiyonları tedavi etme ve deri hastalıklarında kullanılan bitki türleri antimikrobiyal aktiviteleri bakımından test edilmiştir. Hedera helix, Lavandula stoechas subsp. cariensis, Plantago major, Teucrium chamaedrys subsp. chamaedrys ve Teucrium polium'un topraküstü kısımlarından petrol eteri ve etanol ekstreleri hazırlanmış ve antimikrobiyal aktiviteleri 7 bakteri ve bir mantar suşuna karşı CLSI'ya göre dilüsyon tekniği kullanılarak in vitro olarak test edilmiştir. Sonuçlar L. stoechas subsp. cariensis ve P. major etanol ekstrelerinin Staphylococcus aureus'a karşı 19.52 µg/ml MİK değeri ile en kuvvetli aktiviteyi gösterdiğini belirtmektedir. T. polium ve T. chamaedrys subsp. chamaedrys petrol eteri ekstreleri Klebsiella pneumoniae'ye karşı orta derecede aktivite (MİK: 156.2 µg/ml) göstermiştir. Ekstrelerin hiçbiri Candida albicans'a karşı antifungal aktivite göstermemiştir.

Anahtar kelimeler: *Geleneksel ilaçlar, Bilecik (Türkiye), Antimikrobiyal aktivite, Bitki.*

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INTRODUCTION

Bilecik is a province in midwest Turkey, neighboring Bursa to the east, Kocaeli and Sakarya to the north, Bolu to the west, Eskişehir to the southeast and Kütahya to the south, spanning an area of 4307 km^2 (Figure 1). The region was inhabited as early as 3000 BC, and was part of the territory controlled by such notable civilizations as the Hittites, Lydians, Persians, Romans and Byzantians. The region is also where the Ottoman Empire was founded in 1281, and is the source of important archaeological as well as cultural artifacts. Osmaneli is a district of Bilecik province and situated on a hillside at a point where the Karasu, flowing down from Kandilli Dağ enters the Sakarya. South of the town is a narrow and very beautiful river gorge with rocky walls up to 100 m contributing to some spectacular scenery. As a result of rich flora and a variety of different cultures a rich tradition of folk medicine is expected.

Traditional medicine of Bilecik province has been reported for the first time and five species of plants namely *Hedera helix* L. (Araliaceae), *Lavandula stoechas* L. subsp. *cariensis* (Boiss.) (Lamiaceae), *Plantago major* L. (Plantaginaceae), *Teucrium chamaedrys* L. subsp. *chamaedrys* (Lamiaceae), and *Teucrium polium* L. (Lamiaceae) having traditional claims for the treatment of various skin disorders were investigated for their antimicrobial activities.

Figure 1. The map of Bilecik

EXPERIMENTAL

Ethnopharmacological investigations

Ethnopharmacological interviews were conducted in three field trips carried out in June 2003, September 2003 and in May 2004. During the three months field study 6 districts and within these districts 10 villages were visited. The name of the districts and 10 villages were given below (in Table 1) with the reference number for the record place of the local information:

Table 1. Reference numbers of the districts and villages

Reference number	District	Village
1	Osmaneli	
2		Hıdrellez location
3		Balçık-Hisaryolu
4		Selçik village
5	Yenipazar	
6	Pazaryeri	
7		Dereköy
8		Küçükelmalı village
9	Bozüyük	Alibeydüzü village
10		Günyarık village
11	Gölpazarı	Üyük village
12		Kurşunlu village
13	Söğüt	Kepen

A total of 10 informants were interviewed using a semi-structured interview form include questions on vernacular name, plant part, locality, medicinal uses of plant, local traditional prescriptions, preparations and route of administration. The various data about the traditional medicine were obtained from older people in the villages, local healers, experienced adults and patients by personal interviews.

Voucher herbarium specimens were deposited in the Collection of the Scientific and Technical Research Center of Traditional Medicine, Istanbul University (GIM). The identification was carried out by Prof. Asuman Baytop (Department of Pharmaceutical Botany, Faculty of Pharmacy, Istanbul University).

Extract preparation and antimicrobial activity

20 g dried plant material was powdered and extracted with petroleum ether (Merck) and ethanol (Tekel®) successively by soxhlet extraction. The antimicrobial activity of selected plant species has been determined by the microbroth dilutions technique using CLSI recommendations (1, 2).

The extracts were dissolved in dimethylsulfoxide (DMSO, 10 mg/ml) prior to the antimicrobial activity test.

The standard strains used were *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 8739, *Klebsiella pneumoniae* ATCC 4352, *Proteus mirabilis* ATCC 14153, *Pseudomonas aeruginosa* ATCC 1539, *Shigella flexneri* and *Candida albicans* ATCC 10231.

Mueller-Hinton broth for bacteria, RPMI-1640 medium for yeast strain were used as the test medium. Serial two-fold dilutions ranging from 5000 µg/ml to 4.9 µg/ml were prepared in medium. The inoculum was prepared using a 4-6 h broth culture of each bacteria and 24 h culture of yeast strains adjusted to a turbidity equivalent to a 0.5 Mc Farland standard, diluted in broth media to give a final concentration of 5×10^5 cfu/ml (colony-forming units per milliliter) for bacteria and 0.5×10^3 to 2.5×10^3 cfu/ml for yeast in the test tray. The trays were covered and placed in plastic bags to prevent evaporation. The trays containing Mueller-Hinton broth were incubated at 35°C for 18-20 h and the trays containing RPMI-1640 medium were incubated at 35°C for 46-50 h. The MIC was defined as the lowest concentration of compound giving complete inhibition of visible growth.

RESULTS AND DISCUSSION

Results obtained from field surveys are summarized in Table 2. The plant species are included in this table with scientific names, family names, vernacular names, parts of the plant used, usage, preparation, administration, voucher herbarium specimen number (GIM No.) and district (3,4). The folkloric usage of the plants were compared with published data and the results obtained were evaluated.

In this study, information about folk medicine was collected for 64 plant species from 33 families. Of 64 samples 47 had similar usages as in the literature whereas 10 had not. The most used families were Asteraceae and Lamiaceae. A total of 26 medicinal uses were obtained. Plants are used mainly for gastrointestinal disorders (18%), antirheumatismal and analgesic (15%), diuretic (11%), respiratory disorders (11%) and psychological disorders (9%). Medicinal uses of *Cionura erecta, Echium plantagineum, Neslia apiculata, Stachys thirkei, Oxalis articulata, Consolida regalis* subsp. *paniculata* and *Solanum alatum* as traditional medicine have been reported for the first time in this study.

Antimicrobial activity results indicate that the ethanolic extracts of *Lavandula stoechas* subsp. *cariensis* (MIC: 19.52 µg/ml) and *Plantago major* (MIC: 19.52 µg/ml) have shown the strongest activity against *Staphylococcus aureus*. The petroleum ether extract of *L. stoechas* subsp. *cariensis* has also exhibited a significant activity against *S. aureus* with a MIC value of 39.06 µg/ml. In contrast to our study, no activity was observed on the petroleum ether and ethanolic extracts of *L. stoechas* which was collected from Sakarya province of Turkey (5). Although the lavender hydrosols and aqueous foliage extracts did not have any antibacterial activity, it is reported that *L. stoechas* essential oils had low levels linalool and good antibacterial activity (6). It is known that *P. major* leaves have been used in folk medicine for centuries in almost all parts of the world for its wound healing activity and it is reported that 50% ethanol extracts of *P. major* leaves were active against *S. aureus* (7).

Compared with the extracts of *Lavandula*, *Plantago* and *Hedera*, the petroleum ether extracts of *Teucrium polium* (MIC: 156.2 µg/ml) and *T. chamaedrys* subsp. *chamaedrys* (MIC: 156.2 µg/ml) have exhibited moderate activity only against *Klebsiella pneumoniae*. Similar to our result, *T. polium* subsp. *aurasiacum* was also found to be active against *K. pneumoniae* previously (8). But in a published report on the antimicrobial activity of *T. chamaedrys* subsp. *chamaedrys* which was collected from Gümüşhane in the northeastern part of Turkey, the essential oil of this species showed antibacterial activity against *E. coli* and *S. aureus* but no activity against *K. pneumoniae* (9) .

None of the extracts have shown antifungal activity against *C. albicans*.

Table 2. Plant species used as folk medicine in Bilecik province

Scientific name (Family)	Local name	Parts used	District	Use, preparation and administration (GIM No)
Pistachia terebinthus L. subsp. *palaestina* (Anacardiaceae)	Menengiç, Melengiç	Fruits	2	Astringent, chronic bronchitis: Fruits are eaten 2 or 3 times a day Asthma: Smoked as a cigarette (658)
******Rhus coriaria* L. (Anacardiaceae)	Titre, Tetre	Aerial parts	5	Eczema: Cold infusion is consumed 3 times a day (611)
Echinophora tenufolia L. subsp. *sibthorpiana* (Apiaceae)	Tarhana otu	Aerial parts	1	Galactagogue: Infusion is consumed 3 times a day (651)
Foeniculum vulgare Miller (Apiaceae)	Yabani rezene	Seeds and fresh plants	2	Carminative: Infusion is consumed 3 times a day (656)
Hedera helix L. (Araliaceae)	Kaya sarmaşığı	Leaves	2	Antihelmintic and cathartic: Infusion is consumed 3 times a day Inflamed wounds: Fresh leaves cover infected skin (652)
*****Cionura erecta* (L.) Griseb. (Asclepiadaceae)	Panzehir otu	Roots	1	Emetic in case of poisining: Infusion prepared from the roots (0.5 g) is consumed as tea 3 times a day (638)
******Achillea crithmifolia* Waldst. ex. Kit. (Asteraceae)	Civan perçemi	Aerial parts	6	Pain in gastrointestinal system: Infusion is consumed as tea (618)

Artemisia absinthium L. (Asteraceae)	Pelinotu	Aerial parts	1	Appetizer, antihelmintic and carminative in children: Infusion is consumed as tea, or fresh leaves are mixed with honey consuming 3 times a day (619)
Calendula officinalis L. (Asteraceae)	Aynısafa	Flowering tops	1	Wound healing: Infusion is consumed as tea (662)
Chondrilla juncea L. var. *juncea* (Asteraceae)	Karakavut	Mastix	3	Pain in stomach: Latex obtained from the trunk is chewed as gum (646)
****Cichorium intybus*** L. (Asteraceae)	Yabani hindiba	Roots and leaves	1	Liver diseases: Infusion prepared from the root is consumed as tea. Leaves are used as food (609)
Matricaria chamomilla L. (Asteraceae)	Papatya	Flowers	1	Mild sedative: Infusion is consumed as tea (667)
Tanacetum vulgare L. (Asteraceae)	Solucan otu	Aerial parts	1	Antihelmintic: Decoction is consumed as tea (660)
Anchusa undulata L. *ssp. hybrida* (Ter.) Coutinho (Boraginaceae)	Sığırdili	Aerial parts	1	Diaphoretic: Decoction is consumed as tea (628)
***Echium plantagineum* L. (Boraginaceae)	Engerek otu	Aerial parts	1	Diaphoretic and diuretic: Decoction is consumed as tea (606)
Heliotropium europaeum L. (Boraginaceae)	Akrep otu	Aerial parts	1	Against snake and scorpion bite: Decoction is consumed as tea (629)
Capsella bursa-pastoris (L.) Medik. (Brassicaceae)	Çoban çantası	Aerial parts	1	Diuretic and haemostatic: Decoction is consumed as tea (664)
***Neslia apiculata* Fisch. Mey.&Ave-Lall (Brassicaceae)	Hardal	Aerial parts and seeds	1	Rheumatism: Mashed seeds with water are applied to painful area (623)
Humulus lupulus L. (Cannabinaceae)	Şerbetçiotu	Aerial parts	7	Diaphoretic and diuretic: Infusion is consumed as tea. Insomnia: Pillow filled with dried strobiles is used (630)

Capparis spinosa L. (Capparidaceae)	Kedi tırnağı, Kebere	Flower buds	1	Appetizer and tonic: Infusion consumed as tea (647)
Cistus laurifolius L. (Cistaceae)	Tavşanak	Leaves	4	Antidiabetic: Decoction is consumed as tea (631)
Cistus salviifolius L. (Cistaceae)	Laden	Leaves	1	Expectorant: Infusion is consumed as tea (663)
Equisetum arvense L. (Equisetaceae)	At kuyruğu	Aerial parts	9	Antilithic, diuretic: Infusion is consumed as tea (617)
Hypericum avicularii Jaub. et. Spach. (Hypericaceae)	Mide otu	Aerial parts	5	Pain in stomach: Infusion is consumed as tea (612)
Hypericum perforatum L. (Hypericaceae)	Sarı kantaron	Aerial parts	1	Ulcer in stomach: Aerial parts are soaked in vegetable oil and consumed (607)
Juglans regia L. (Juglandaceae)	Ceviz	Leaves	2	Lowering blood sugar, appetizer, tonic and astringent: Infusion is consumed as tea (657)
Calamintha nepeta (L.) Savi subsp. *gladulosa* (Req.) P.W. Ball. (Lamiaceae)	Kedi nanesi	Aerial parts	1	Carminative: Infusion is consumed as tea (636)
Lavandula stoechas L. subsp. *cariensis* (Boiss.) Rozeira (Lamiaceae)	Karabaş otu	Flowers	1	Wound healing: Infusion is consumed as tea (668)
Lycopus europaeus L. (Lamiaceae)	Kurt ayağı	Aerial parts	4	Haemostatic, antipyretic: Decoction is consumed as tea (659)
Melissa officinalis L. subsp. *altissima* (Sm.) Arcangeli (Lamiaceae)	Oğulotu	Leaves	1	Insomnia, mild sedative, against cardiac problems: Infusion is consumed as tea (622)
Mentha longifolia (L.) Hudson subsp. *typhoides* (Briq.) Harley var. *typhoides* (Lamiaceae)	Eşek nanesi	Leaves	2	Carminative: Infusion is consumed as tea (657)
Mentha piperita L. (Lamiaceae)	Nane	Leaves	11	Cold and cough: Infusion is consumed as tea (597)

Ocimum basilicum L. (Lamiaceae)	Reyhan	Leaves	1	Appetizer , depurative: Infusion is consumed as tea (634)
Origanum hirtum Link. (= *O. heracleoticum* L.) (Lamiaceae)	Mercanköşk	Aerial parts	1	Pain in belt: Volatile oil is used externally (601)
Salvia aethiopis L. (Lamiaceae)	Adaçayı	Leaves	1	Cold: Infusion is consumed as tea (608)
Salvia sclarea L. (Lamiaceae)	Adaçayı	Leaves	1	Cold: Infusion is consumed as tea (602)
**Stachys thirkei* C. Koch. (Lamiaceae)	Kestere	Aerial parts	1	Gastrointestinal disorders: Decoction is consumed as tea (599)
***Teucrium chamaedrys* L. subsp. *chamaedrys* (Lamiaceae)	Kısamahmut otu	Aerial parts	1	Ulcer in mouth, kidney infection: Infusion is consumed as tea (605)
Teucrium polium L. (Lamiaceae)	Acı yavşan	Aerial parts	1	Pain in stomach, carminative, wound healing and appetizer (603)
Thymbra spicata L. (Lamiaceae)	Kara kekik	Aerial parts	1	Stomachic: Infusion is consumed as tea (604)
***Epilobium parviflorum* Schreber (Onagraceae)	Yakı otu	Aerial parts	2	Astringent, used in haemorrhoids: Decoction is consumed as tea (653)
Anacamptis pyramidalis (L.) L.C.M. Richard (Orchidaceae)	Salep	Cormus	1	Aphrodisiac and tonic: Powdered drug is mixed with milk (669)
**Oxalis articulata* Savigny. (Oxalidaceae)	Ekşi yonca	Leaves	1	Antiinflammatory: Fresh leaves are eaten or its juice is dropped in sugar to eat (654)
Chelidonium majus L. (Papaveraceae)	Kırlangıç otu	Latex	1	Wart: The latex is applied on a wart (639)
Glycyrrhiza glabra L. var. *glandulifera* (Waldst. et. Kit.)	Meyan	Roots	1	Stomachic, sore throat: Decoction is consumed as tea (632)

Boiss. (Papilionaceae)				
Psoralea bituminosa L. (Papilionaceae)	Katran yoncası	Leaves	1	Mild sedative: Decoction is consumed as tea (661)
Phytolacca americana L. (Phtolaccaceae)	Şekerci boyası	Fruits, roots, leaves	1	Cathartic: Decoction is consumed as tea (663)
Plantago major L. (Plantaginaceae)	Beyaz bıçak otu	Leaves	8	Acne, wound healing: Boiled and mashed leaves are applied on skin (627)
******Plumbago europaea* L. (Plantaginaceae)	Sıtma otu	Flowering tops	1	Rubefacient, antipyretic: Flowering branches are applied on skin (644)
*****Consolida regalis* S.F.Grey subsp. *paniculata* (Host) Soo var. *paniculata* (Ranunculaceae)	Tarla hezareni, altıntop	Flowers	3	Migrain: Onespoonful decoction is consumed 2-3 times a day (643)
Helleborus orientalis Lam. (Ranunculaceae)	Bohça otu	Roots	13	Insect repellent: Piece of root is inserted on ear skin of cattle (613)
Reseda lutea L. (Resedaceae)	Muhabbet çiçeği	Aerial parts	1	Diaphoretic and diuretic: Decoction is consumed as tea (635)
Crataegus tanacetifolia (Lam.) Pers. (Rosaceae)	Alıç	Flowers	10	Antihypertensive: Infusion is consumed as tea (616)
Rosa canina L (Rosaceae)	Köpek dikeni	Fruits	13	Blood sugar lowering, bronchitis: Infusion is consumed as tea (614)
******Galium verum* L. (Rubiaceae)	Yoğurt otu	Flowering tops	1	Sore throat and guatr: Decoction is consumed as tea (645)
******Ruta montana* (L.) L. (Rutaceae)	Yabani sedefotu	Aerial parts	1	Antihelmintic: One teaspoonful decoction is consumed 2-3 times a day
Ailanthus altissima (Miller) Swingle (Simaroubaceae)	Kokarağaç	Rinds of trunk	1	Antispasmodic, astringent, emetic: Infusion is consumed as tea (640)
******Lycium chinense* Miller	Teke dikeni	Flowering tops, roots	1	Stimulating kidney and liver: Decoction is

(Solanaceae)		and fruits		consumed as tea (655)
Physalis alkekengi L. (Solanaceae)	Güvey feneri	Fruits	1	Diuretic and tonic: Fresh fruits are eaten or decoction is consumed as tea (621)
**Solanum alatum* Moench. (Solanaceae)	Köpek üzümü	Leaves and fruits	1	Analgesic, sedative, haemorrhoids: Decoction is consumed as tea or mashed and boiled drug is applied on haemorrhoides (641)
Urtica dioica L. (Urticaceae)	Isırgan	Aerial parts	12	Rheumatism: Fresh plant is applied on painful area (596)
Valeriana dioscorides Sm. (Valerianaceae)	Kedi otu	Roots	1	Sedative, insomnia: Infusion is consumed as tea (666)
***Verbena officinalis* L. (Verbenaceae)	Mine çiçeği	Aerial parts	2	Astringent, antipyretic: Decoction is consumed as tea (648)
Peganum harmala L. (Zygophyllaceae)	Üzerlik	Seeds	4	Sedative, antihelmintic, diaphoretic: Infusion is consumed as tea. Roasted seeds are used against haemorrhoids (650)

*Traditional uses of these plants have been reported for the first time in this work
**Traditional uses of these plants have been found different from the literature findings (4).

Table 3. Minimum inhibitory concentrations of extracts from selected species (μg/ml)

			Microorganisms[c]							
Species and Reference compound	Plant part[a]	Extract[b]	*S.au.*	*S.epid.*	*E.coli*	*K.pne.*	*P.mir.*	*P.ae.*	*S.flex.*	*C.alb.*
H. helix	L	PE	312.5	625	1250	1250	1250	Na	1250	625
		ETH	156.2	625	1250	1250	1250	Na	2500	625
L. stoechas	F	PE	39.06	156.2	1250	1250	1250	Na	625	625
		ETH	19.52	312.5	1250	1250	1250	Na	1250	625
P. major	L	PE	156.2	312.5	1250	1250	1250	Na	625	625
		ETH	19.52	625	1250	1250	1250	Na	2500	625
T. chamaedrys	AP	PE	625	1250	1250	156.2	Na	Na	1250	1250
		ETH	Na	1250	1250	625	Na	1250	1250	2500
T. polium	AP	PE	Na	1250	Na	156.2	Na	1250	1250	Na
		ETH	2500	1250	Na	625	Na	1250	1250	2500
Cefuroxime-Na			1.2	9.8	4.9	4.9	2.4	—	4.9	—
Ceftazidime			—	—	—	—	—	2.4	—	—
Clotrimazole			—	—	—	—	—	—	—	4.9

[a]AP, aerial parts; F, flowers; L, leaves.
[b]PE, petroleum ether; ETH, ethanol.
[c]S.au., *Staphylococcus aureus*; S.epid., *Staphylococcus epidermidis*; E.coli, *Escherichia coli*; K.pne., *Klebsiella pneumoniae*; P.mir., *Proteus mirabilis*; P.ae., *Pseudomonas aeruginosa*; S.flex., *Shigella flexneri*; C.alb., *Candida albicans*; NA, not active; —, not tested

ACKNOWLEDGEMENT

The authors are grateful to Prof.Dr. A. Baytop for identification of the plant materials.

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Received: 02.07.2009
Accepted: 15.10.2009