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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

In Vitro Anti-*Helicobacter pylori* and Antimycobacterial Activity Evaluation of Selected Plants From Turkey

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Abstract: In this study, the extracts of *Ulmus minor* Mill. subsp. *minor* (Ulmaceae), *Lathyrus pratensis* L. (Leguminosae), *Glaucium leiocarpum* Bois. (Papaveraceae), and *Echium vulgare* L. (Boraginacea) were investigated for their *in vitro* anti-*Helicobacter pylori* and anti-mycobacterial activity.
The air-dried plant material was powdered and extracted with 70% ethanol by maceration. The extract was filtered and evaporated to dryness under the vacuum and then dissolved in a water-ethanol (90:10) mixture and extracted with dichloromethane and ethyl acetate, respectively. Antibacterial activity was investigated by microdilution method against *Helicobacter pylori* ATCC 43504, *Mycolicibacterium smegmatis* ATCC 14468 and *Mycobacterium avium* ATCC 25291.
The ethyl acetate extract of *U. minor* showed activity with MIC of 250 μg / mL against *H. pylori*. The other extracts showed no or weak inhibitory activity at >2000 μg/mL concentrations against the tested microorganisms. In conclusion, ethyl acetate extract of *U. minor* may be used for the treatment of *H. pylori* infections.

Keywords: *Echium, Glaucium, Helicobacter pylori, Lathyrus, Mycobacterium, Ulmus.*

Türkiye'de Yetiştirilen Bazı Bitkilerin *İn Vitro* Anti-*Helikobakter pilori* ve Antimikobakteriyel Aktivitesi

***Sorumlu yazar:**
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Öz: Bu çalışmada, *Ulmus minor* Mill. subsp. *minor* (Ulmaceae), *Lathyrus pratensis* L. (Leguminosae), *Glaucium leiocarpum* Bois. (Papaveraceae) ve *Echium vulgare* L. (Boraginacea) ekstrelerinin antihelikobakter pilori ve antimikobakter aktiviteleri *in vitro* olarak incelenmiştir. Kurutulmuş bitki materyalleri toz edildikten sonra %70'lik etanolle masere edilmiştir. Ekstreler süzülerek vakum altında yoğunlaştırıldıktan sonra su-etanol (90:10) karışımında çözülerek sırasıyla diklorometan ve etilasetat ile alt ekstreleri hazırlanmıştır. Antibakteriyel aktivitesi *in vitro* broth mikrodilüsyon yöntemi ile *Helicobacter pylori* ATCC 43504, *Mycolicibacterium smegmatis* ATCC 14468 ve *Mycobacterium avium* ATCC 25291'a karşı araştırılmıştır.
U. minor'un etilasetat ekstresinin MİK değeri *H. pilori*'ye karşı 250 µg/mL olarak kaydedilmiştir. Test edilen konsantrasyonlarda (2000 µg/mL) diğer ekstreler belirgin bir MİK değeri göstermemiştir. Sonuç olarak *U. minor* yapraklarının etilasetat ekstresinin *H. pilori* enfeksiyonlarında tedavi edici olarak kullanılabileceği düşünülmektedir.

Anahtar kelimeler: *Echium, Glaucium, Helikobakter pilori, Lathyrus, Mikobakter, Ulmus.*

INTRODUCTION

Mycobacterium species can be found in soil, water, dust particles, domestic or wild animals, milk and other nutrients and can be transmitted to humans from the environment. They can be colonized on body surface and secretions. *Mycobacterium avium* complex causes various ailments in humans and animals (Thorel et al., 2001). *Mycobacterium avium* complex contains the factors of *M. avium* subsp. *paratuberculosis*. The agent of *M. paratuberculosis* causes ruminant and other mammal species called Johne disease or paratuberculosis (Biet et al., 2005; Hoop, 2002; Tell et al., 2001). *Mycolicibacterium smegmatis* is a rapid-growing bacterium and it is used instead of *M. tuberculosis* and *M. leprae* in studies (Bashiri &Baker, 2005).

Ulmus minor Mill. subsp. *minor* belongs to the Ulmaceae family and this family represented by three species in Turkey (Davis, 1982). Also, there are different *Ulmus* species around the world such as, Britain, Japan, Korea, China (Armstrong et al., 1996; Choi et al., 2010). There are no medicinal uses of *U. minor* subsp. *minor* in Turkey. However, the bark of the root and stem of *U. davidiana* var. *japonica* has been used as a traditional Korean medicine. It is used to treat inflammatory disorders and exhibits antioxidant, anticancer, and anti-inflammatory effects (Choi et al., 2010). To treat alopecia; some galls which are found on the branch and stem of the tree are cut and the juice and worms inside the galls are applied to the affected area of the head. The treatment should be repeated for several consecutive day. For scabies; the thin layer under the bark of branches (called 'sirri') is peeled, boiled with water until thick, then applied to the affected part (Yeşilada et al., 1999). Moreover, leaves of *U. minor* are used in veterinary treatment to cure digestion problems of rabbits and ovines in traditional usage in Tuscany (Italy) (Manganelli et al., 2001) *Ulmus* species contain biologically active compounds, such as sesqui-terpenoids, triterpenes and flavonoids (Lee et al., 2008; Zheng et al., 2010).

Lathyrus L. (Fabaceae) genus has more than 160 species and *Lathyrus* species used different uses, such as alimentary, agricultural, industrial, ornamental, and in traditional medicine worldwide (Lee et al., 2008; Zheng et al., 2010). For instance, *L. odoratus* and *L. sativus* are used for agricultural processes in Turkey and *L. paratensis* showed moderate to strong antifungal bioactivity (Arabi & Sardari, 2010). Moreover, in traditional medicine, it is used for different purposes such as analgesic (seed of *L. sativus*), anti-inflammatory (aerial parts of *L. cicera*), and anti-rheumatism (leaf of *L. rotundifolius* Willd. subsp. miniatus (Bieb. ex Stev.) Davis) in Turkey (Llorent-Martínez et al., 2017). Additionally, Holbrook et al. stated that many species contain novel amino acids and nitriles that are toxic. Though the toxicants are present in vegetative parts of the plant, they are more concentrated in the seeds. Lathyrism can be seen in humans and various animals such as horses, cattle, sheepdogs, rabbits, rats and mice (Holbrook et al., 2015).

Glaucium genus represented by 7 species in Turkey (Cullen, 1965). These species are rich in alkaloid content. *Glaucium leiocarpum* Boiss. (Papaveraceae) is widely distributed in Greece, Crete, Iran, Syria, Caucasia and Turkey, mostly on roadsides, stony fields and slopes. *Glaucium* genus' have antimicrobial, antiinflammatory, antitumoral and analgesic activity and potency to cure age-related brain disorders and it is also shown that *Glaucium* species have been used in Iranian and Turkish herbal medicine as laxative, antidiabetic, hypnotic, antifungal and for treatment of dermatitis (Baytop, 1984; Bournine et al., 2013; Hakemi et al., 2017; Morteza-Semnani et al., 2003; Orhan et al., 2004; Soureshjan & Heidari, 2014). G. *leiocarpum* named ''Kuş ekmeği'' in Aydınlar Village (Erdemli/Mersin) and it has been used in traditional medicine as soothing, cough suppressant, intestinal softener and for culinary purposes (Eşen, 2008).

Echium vulgare L. (Boraginaceae) is originated in the Mediterranean and naturalized in Africa, South and North America, Canada, Asia (Japan), Europe (United Kingdom, Turkey), New Zeland and Australia (Klemow et al., 2002). *Echium* species contain pyrrolizidine alkaloids, naphthoquinones (such as shikonins). Shikonins exhibit potent antimicrobial, antifungal, anticonvulsive effect and phytotoxic properties and are frequently used as biomedicinals in Eastern medicine (Kelmow et al., 2002; Papageogiou et al., 1999; Parsons et al., 2001; Zhu et al., 2016). Moreover, *E. vulgare* leaves are used against dermatosis to traditionally cure animals in Tuscany (Italy) (Manganelli et al., 2001). Multiple sources recommend that the plant be taken internally as a tea, powder, tincture, or medicinal wine. Moreover, it is known that *E. vulgare* used for treating urinary tract disorders and as an aid in childbirth in North America. Although prolonged internal use of *E. vulgare* may be cause to hepatotoxicity (Klemow et al., 2002).

In this study, antimicrobial activity of the selected four plants against *Helicobacter pylori* and Mycobacter strains was investigated to find an alternative and natural solution to the microbial disease such as stomach disorders or Mycobacterial infections which caused by these microorganisms.

MATERIAL AND METHOD

Collection of plant material: The plant materials of *Lathyrus pratensis* L, *Ulmus minor* Miller ssp. *Minor*

and *Echium vulgare* L. were collected in their flowering periods from Giresun-Şebinkarahisar. *Glaucium leiocarpum* Boiss. were collected from Ankara- Çankaya. Voucher specimens were deposited in the Herbarium of Istanbul Medipol University, School of Pharmacy, Department of Pharmacognosy.

Solvent extraction: The air-dried plant material was powdered and extracted with 70% ethanol by maceration. The extract was filtered and evaporated to dryness under reduced pressure in a rotary evaporator. The concentrated residue dissolved in a water-ethanol (90:10) mixture and then extracted with liquid-liquid extraction by dichloromethane (DCM) and ethyl acetate (EtOAc), respectively (Karadağ & Tosun, 2019; Acet, 2019).

Antimicrobial Activity: The antimicrobial activity was studied using the broth microdilution assay by a modified Clinical and Laboratory Standards Institute protocol (CLSI) (Whitmire & Merrell, 2012). All microorganisms were standardized versus McFarland No: 0.5 in sterile saline (% 0.85) turbidimetrically. The stock solutions of the extracts were dissolved in dimethylsulfoxide (DMSO), and serial dilutions were prepared. Diluted bacterial suspensions were added to each well and then incubated at 37 °C for twenty-four hours.

Helicobacter pylori ATCC 43504 were grown for 24 hours in Brucella broth containing 5% (v/v) horse blood Colombia agar and containing 10% (h/h) fetal bovine serum (FBS) at 37 °C in an anaerobic incubator (5% CO_2). 100 μL of 1:10 diluted and density modulated *H. pylori*'s strain was added to each microplate. The MICs were calculated as the mean of three repetitions, which are reported in Table 1.

Mycobacterium strains were inoculated in Middlebrook 7H11 agar (Sigma Aldrich), and incubated in aerobic conditions at 37 °C for 4-5 days. The microorganism was transferred to the cation doped MHB and incubated for a further five days. Growing cultures were vortexed and allowed to collapse for 30 min. Diluted bacterial suspensions (10^6 CFU/mL) were added to each well and then allowed to incubate at 37 °C for 5 days. Amoxicillin, clarithromycin and tetracycline were used as positive control and DMSO used as negative control (Karadağ & Tosun, 2019).

RESULTS AND DISCUSSION

Eight extracts were tested against the pathogenic bacteria listed in Table 1. Using a modified micro-broth dilution assay, MIC values of extracts were compared with those of amoxicillin, clarithromycin, and tetracycline. DMSO was used as negative control. 2000-62.5 µg/mL concentration range was studied for determine the MIC value. Among the tested bacteria in this present study, *H. pylori* was the more sensitive to the *U. minor* EtOAc extract (250 µg/mL), while *Mycobacteria* appeared to be the most resistant (>2000 µg/mL). While a moderate antimicrobial activity was observed in *U. minor* extracts against *H. pylori* strain none of the other extracts showed activity against *Mycobacterium* species in the tested concentrations (i.e., 2 µg / mL).

Table 1. Results of antimicrobial activity determined by microdilution method of samples (µg / mL) and antimicrobials (µg / mL).

Samples	*H. pylori*	*M. smegmatis*	*M. avium*
G. leiocarpum DCM extract	1000	>2000	>2000
G. leiocarpum EtOAc extract	>2000	>2000	>2000
E. vulgare DCM extract	>2000	>2000	>2000
E. vulgare EtOAc extract	>2000	>2000	>2000
U. minor DCM extract	>2000	>2000	>2000
U. minor EtOAc extract	250	>2000	>2000
L. pratensis DCM extract	>2000	>2000	>2000
L. pratensis EtOAc extract	1000	>2000	>2000
Amoxicillin	≤0.125	NE	NE
Clarithromycin	25	64	8
Tetracycline	25	NE	NE

**NE: No effect*

Although here are not many antimicrobial activity studies on the plants used in the study, there is a study showing that *G. leiocarpum* has an ethnobotanical use against wound healing (Bulut et al., 2017). However, antimicrobial activity of some secondary metabolites of *E. vulgare* was investigated, but efficacy was not observed at the tested concentrations (solutions of the pure compounds, solved in MeOH, c 1⁄4 1 mg=ml) against the tested microorganisms (*Escherichia coli, Bacillus subtilis, Staphylococcus aureus and Candida albicans*) (Kuruüzüm-Uz et al., 2004). In an antimicrobial activity screening study on *Lathyrus* species, the MIC value of ethyl acetate extract obtained from *L. pratensis* against the *Staphylococcus aureus, Bacillus subtilis, Escherichia coli,* and *Pseudomonas aeruginosa* and antifungal activity against *Candida albicans* were calculated as 0.5 mg / mL (Heydari et al., 2019). However, MIC values could not be calculated at the concentrations against the microorganisms used in this study.

CONCLUSION

In previous studies, antimicrobial activity of different extracts of *U. minor* stems and leaves were studied and various MIC values are obtained against *Enterecoccus faecalis* and *Salmonella typhii* (Tag et al., 2011) Leaves of *U. minor* is use in veterinary treatment to cure digestion problems of rabbits and ovines in traditional usage in Tuscany (Italy) (Manganelli et al., 2001).

As a result, it can be thought that *U. minor* leaf extracts which are prepared by polar solvent such as methanol, ethanol, water etc. can be used as a supplementary to prevent stomach ulcer caused by *H. pylori*. In addition, this study can be evaluated in future studies to determine active compounds from EtOAc extract of *U. minor* leaves.

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