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Antibacterial and Antioxidant Activity of Traditional Medicinal Plants from the Balkan Peninsula

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ABSTRACT

Negative effects of available antibiotics and the constant development of bacterial resistance motivate a search for new antimicrobial agents. Aromatics plants have traditionally been used as antibacterial agents and are well accepted today as a source of antioxidants. The present study evaluated the antibacterial activities and antioxidant capacity of eight aromatic plants, indigenous to the flora of the Balkan Peninsula, which are used as medicinal plants in traditional medicine. The plants studied were *Hyssopus officinalis*, *Angelica pancicii, Angelica sylvestris, Laserpitium latifolium*, *Achillea grandifolia, Achillea crithmifolia, Artemisia absinthium* and *Tanacetum parthenium*. The antimicrobial activities of methanolic extracts of the plant tissues against 16 bacterial isolates of *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella sp.*, *Proteus mirabilis*, *Acinetobacter sp.*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae* and *Enterococcus faecalis* were investigated using a microwell dilution assay. Minimal inhibitory concentration (MIC) of the extracts ranged from 6.3 to 100 mg mL^{-1}, and minimal bactericidal concentration (MBC) ranged from 12.5 to 100 mg mL^{-1}. Antioxidant potential of the extracts was analyzed as contents of total phenols and flavonoids; radical scavenging activity by the $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ methods, and reducing power by the iron (III) to iron (II) reduction assay, and the ferric reducing antioxidant power assay (FRAP). Results of antioxidative activities from the 4 methods demonstrated similar sequence of activity: *A. crithmifolia* > *A. grandifolia* > *H. officinalis* > *A. absinthium* > *T. parthenium* > *L. latifolium* > *A. pancicii* > *A. sylvestris*. The total content of polyphenols and flavonoids in the methanol extracts of the studied species positively correlated with their antioxidant properties, confirming their major role in antioxidant activity of these species.

1. Introduction

Infectious diseases are a major cause of morbidity and reduced mortality particularly in developing countries. A wide range of synthetic and semi-synthetic antibacterial agents is available today for the control of microorganisms; however resistance of bacteria to the available antibacterial agents is growing rapidly [1,2]. Along with the beneficial effects of bacterial control, the available antibiotics also cause various adverse drug reactions such as hypersensitivity and immunosuppression [3,4]. Due to these negative effects, and the constant development of bacterial resistance, there is a continuous need to develop newer antimicrobial agents effective against microorganisms and less harmful to the host. Therefore, the pharmaceutical industry is motivated to develop alternative antimicrobial drugs.

One of the most significant natural sources of antimicrobial agents are essential-oil containing aromatic-plants, many of which are used in traditional medicine primarily to combat infectious diseases [5]. Plants containing natural products have been used worldwide in traditional medicine since antiquity [6] and are a source of potential and powerful drugs [7]. The potential of higher plants as a source for new drugs is still largely unexplored, and among the estimated 250,000- 500,000 plant species, only a small fraction has been submitted to biological or pharmacological

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Table 1
Information about the right aromatic plants evaluated in the study: plant part collected for the analyses; phase of plant development at the time of sampling; location and habitat of the collection zone. All the collection sites are located in East Serbia. I, inflorescence.

Species	Plant part	Phase of plant development	Habitat	Location of collection
Achillea grandifolia	Vegetative + I	Flowering	Forest- shady locations	Jerma Gorge, Trnski Odorovci village
Achillea crithmifolia	Vegetative + I	Flowering	Dry meadows	Stara Planina, Crni Vrh village
Angelica pancicii L.	Vegetative + I	Flowering	River banks	Vlasina plateau, Crna Trava village
Angelica sylvestris L.	Vegetative + I	Flowering	Wet meadows; swamps	Dimitrovgrad, Mazgoš village
Artemisia absinthium	Vegetative + I	Flowering	Road sides	Niška Banja, Malča village
Hyssopus officinalis L.	Vegetative	Vegetative	Dry, rocky	Visočka Ržana, Rsovci village.
Laserpitium latifolium	Vegetative + I	Flowering	High mountain pasture	Stara Planina, Babin Zub peak
Tanacetum parthenium	Vegetative + I	Flowering	Forest edge	Stara Planina, Babin Zub peak

screening. The therapeutic activity of plants is attributed mainly to their antimicrobial activities. Usage of plant-derived antimicrobial agents might be effective in reducing the dependence on antibiotics and minimizing the chances of antibiotic resistance in foodborne pathogenic microorganisms [8].

Aromatic plants are widely used also as nutritional supplements [9]. Of special interest is their value as a source of natural antioxidants [10]. Due to increasing safety concerns involved with consumption of synthetic antioxidants, exploitation of cheaper and safer sources of antioxidants from natural origins, and especially from plants, is of interest nowadays. The major plant compounds characterized by antioxidant activity are polyphenols. The antioxidant activity of polyphenols is accredited to their redox properties [11] i.e., adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, and decomposing peroxides. The most common and widely distributed group of phenolic compounds in plants is flavonoids. These are present in most plants and are considered to prevent free radicals associated damages by numerous ways including direct scavenging of free radicals and inhibition of enzymes involved in free radical production [12].

In the present study we have investigated the antimicrobial potential of metanolic extracts of representatives of the families *Lamiaceae, Apiaceae* and *Asteraceae*, which include the most common aromatic plant species in the Balkan Peninsula and Europe [13,14]. The selected 8 aromatic plants are commonly collected and used in the local traditional medicine at the Balkan Peninsula as antimicrobial agents (*Hyssopus officinalis*; *Angelica sylvestris*; *Angelica pancicii*; *Achillea crithmifolia*; and *Artemisia absinthium*); or against a range of other ailments *Tanacetum parthenium* and *Laserpitium latifolium*); or their chemical composition points at the potential for antimicrobial activity (*Achillea grandifolia*). Methanolic extracts of the selected plants were tested against 16 pathogenic bacteria isolated from human material.

Very little information is available concerning the antibacterial activity of the studied plants. To the best of our knowledge, the activity of methanol extracts of *T. parthenium, A. grandifolia, L. latifolium* and *A. pancicii* have never been studied; methanol extracts from *A. absinthium* [15,16] and *H. officinalis* [17–19] were analyzed only by the disk-diffusion method, which provides qualitative and not always reproducible data [20,21]; and methanol extracts of *A. crithmifolia* and *A. sylvestris* seeds was tested against a limited range of bacteria [22,23].

No information is available also about the antioxidant potential of the selected plants: the antioxidant activity of methanol extracts of *A. pancicii, A. sylvestris*, *A. grandifolia, A. crithmifolia* and *H. officinalis* were never studied (information about ethanol extract is available for *H. officinalis*- Alinezhad et al., [24]); methanol extract of *T. parthenium* [25] and extracts of different polarities of *A. absinthium* [26] were investigated only for the content of polyphenols; daucane sesquiterpenoids (laserpitin and acetyldesoxodehydrolaserpitin) and the phenylpropanoids laserin and latifolon were isolated from chloroform extracts of *L. latifolium* [27]. The methanol extracts were therefore studied also for antioxidant potential. They were analyzed for contents of phenol and flavonoids; antioxidant activity was assessed by scavenging effect on the $ABTS^{\bullet+}$ radical cation and the $DPPH^{\bullet}$ free radical [28]; reducing power, which is considered to be an indicator of antioxidant activity was determined using a modified iron (III) to iron (II) reduction assay, and the ferric reducing antioxidant power assay (FRAP).

2. MATERIAL AND METHODS

2.1. Plant material

Eight aromatic plant species indigenous to the flora of the Balkan Peninsula, and commonly used as medicinal herbs in traditional local medicine, were analyzed in the present study. The 8 selected medicinal plants belonging to the families Lamiaceae, Asteraceae and Apiaceae were: *Hyssopus officinalis* L. (Lamiaceae) (7271), *Achillea grandifolia* (Asteraceae) (7270), *Achillea crithmifolia* (Asteraceae) (7272), *Tanacetum parthenium* (Asteraceae) (7277), *Laserpitium latifolium* (Apiaceae) (7273), *Angelica sylvestris* L. (Apiaceae) (6923), *Angelica pancicii* L. (Apiaceae) (6924) and *Artemisia absinthium* (Asteraceae) (7278). Above-ground parts of the plants were collected during the summer of 2012, from the region of Southeast Serbia. The plant parts collected for the analyses; the phase of plant development at the time of sampling; the specific location of the collection zone and habitat description for each plant are detailed in Table 1. Four independent samples were collected for each species from different plants in a single location. Voucher specimens (acquisition numbers given in brackets near the species name) were deposited in the "Herbarium Moesiacum Niš" (HMN), Department of Biology and Ecology, Faculty of Science and Mathematics, University of Niš.

Hyssopus officinalis is traditionally used for its antiseptic properties in the treatment of infectious disorders. It is known to have antimicrobial, antiviral, carminative and mild spasmolytic effects and its above-ground parts are used as an antiseptic and to treat chronic bronchitis, asthma [29–31]. *Angelica sylvestris* and *A. pancicii* are used as well in traditional medicine as antibacterial agents for the treatments of bronchitis, asthma, influenza and other diseases of respiratory, vascular and digestive systems [29,30,32]. Only little pharmacological information is available for these Angelica species. The root of *L. latifolium* is traditionally used as a diuretic and laxative agent, and its fruits are used for the regulation of appetite and as a digestive agent [33]. *Achillea crithmifolia* is used in traditional medicine for the treatment of gastrointestinal tract conditions, for external use as an antiseptic and an antiphlogistic, while *A. grandifolia* is not commonly used in traditional medicine, but its essential oil chemical profile points at a potential for strong antimicrobial effects [34]. *Artemisia absinthium* is used as a cholagogue, choleretic, carminative and a stomachic agent [30], it exhibits anti-inflammatory and anthelminitic effects, and is used in treating numerous skin conditions [32]. *Tanacetum parthenium* is used in the

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