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ANTIMICROBIAL POTENTIAL OF ROMANIAN SPONTANEOUS FLORA - A MINIREVIEW

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Abstract

In the past decades, clinical microbiologists, practitioners and professionals in food safety, are facing new challenges related to new born microbial pathogens as well as to the phenomenon of the antibiotic and biocide resistance developed by the pathogens. Meanwhile, in Romania has been noticed an increase in scientific publications dealing with the potential of Romanian aromatic and medicinal plants and their therapeutic use. The paper proposes a mini-review on scientifically proved antimicrobial activity of aerial and underground parts of some spontaneous plant from Romanian flora. The review approaches annual and perennial plants, from herbaceous species to bushes and trees. In our search we have identified a total of 64 species from autochthonous flora involved in studies on antimicrobial activity, belonging to 21 botanical families. Among these species, 28.1% are annual plants, 46.9% are herbaceous perennial plants and the rest (25%) are woody perennial species (bushes and trees). Almost 50% of the active species belongs to Asteraceae and Lamiaceae botanical families. For 89% of the species have been reported antibacterial activity, while only 57.8% of the species have proven antifungal activity.

Key words: *Romanian spontaneous flora, antibacterial, antifungal, phytopharmaceutical use.*

INTRODUCTION

In the past decades, clinical microbiologists, practitioners and professionals in food safety, are facing new challenges related to new born microbial pathogens as well as to the phenomenon of the antibiotic and biocide resistance developed by the pathogens, of which the most studied is methicillin-resistant *Staphyloccous aureus* MRSA (Lee et al., 2015). The antibiotic resistance crisis has been attributed to the overuse and misuse of the medications, as well as the lack of new drug development by the pharmaceutical industry due to reduced economic incentives and challenging regulatory requirements (Ventola, 2015). In ancient times, microbial infections were treated empirically, by the use of different natural solutions, including the use of different plants prepared under different formulations. Starting with the discovery of penicillin by Sir Alexander Fleming in 1928, antibiotics have transformed modern medicine. Over the past decades, starting with 1950, it has been proven that bacteria have developed resistance to different antibiotics; as a consequence, new synthesis substances have been developed to treat the infections (Ventola, 2015); because of different mechanisms, more or less elucidated, the microorganisms became over and over resistance to the new antibiotics and biocids. Meanwhile, with the growing consumer demand for natural preservatives to replace chemical compounds, plant antimicrobial compounds must be thoroughly investigated for their potential to serve as natural preservatives (Hintz et al, 2015); on this side, very recently, an exhaustive overview on natural food preservatives with antimicrobial properties has been reported by Pisoschi et al. (2018).In this context, the “return to the origins”, meaning the use of the spontaneous flora as antimicrobial tool may be a solution of the antibiotic “crisis” and the replacement of chemical preservatives. A lot of studies have been published in the past two decades and it has been proven that spontaneous flora of each continent, hemisphere, country or region has an immense potential in obtaining different products with antimicrobial and anti-inflammatory impact. The repository of the whole information is quite difficult because of the huge volume of data, but there are some reports related to different countries or regions. For example, in Balkan regions (Southeast Europe), where Romania belongs, an ethnobotanical analysis showed that 128 plant species (105 wild, 22 cultivated and 1 wild/cultivated) are used in the treatment of wounds. Their application is external, in the form of infusions, decoctions, tinctures, syrups, oils, ointments, and balms, or direct to the skin. Among those plants recorded, the most commonly used in Balkans are *Plantago major, Hypericum perforatum, Plantago lanceolata, Achillea millefolium, Calendula officinalis, Sambucus nigra, Tussilago farfara* and *Prunus domestica* (Jaric et al., 2018). As in all the other countries or regions, on the ancient lands of the actual Romania, it was a vivant interest in the use of different plants to treat human, animal or even plants' infections. Jaric et al. (2018) make references to different studies and report that in Romania, out of more than 3600 species of plants, over 700 are medicinal plants. Nowadays the interest has been resuscitated and more complex studies have been performed related to the chemical composition of the popular plants, as well as on testing their antimicrobial effect by the use of a standardized methodological approach. On our knowledge, there is no comprehensive review on the published studies developed on Romanian level in relation to our local flora and its antimicrobial properties. However, Amarioarei et al. (2016) has published a scientometric analysis performed on data collected from Scopus, during 2000-2015; the review contains information on number of papers, citations, affiliation and number of authors dealing with the potential of Romanian aromatic and medicinal plants and their therapeutic use. The authors have reported an increasing trend for such publications in Romania starting with 2007 which we could rely with the variety of funding available through the national and regional funding programs. Also, there is some information provided from some Romanian geographical regions on plants having different phytopharmaceutical effects; for example, in Banat region were identified about 140 plant species with antioxidant potential (Antal, 2010). Generally, antioxidant activity is due to polyphenol content, especially flavonoids compounds, and it is assumed that same compounds are partially responsible for the antimicrobial activity. The present paper proposes to present an overview of Romanian spontaneous flora proved by experimental approach to have antimicrobial effect.

MATERIALS AND METHODS

Online information research was conducted by the use of different database collections and on-searching engines (Google Academic, Web of Knowledge, PubMed, ScienceDirect and Embase, InTech and Hindawi databases). The information has been structured in relation to the plants type (annual/biennial and perennial plants, from herbaceous species to bushes and trees).Where information was available, the district or the county of the plant origin have been specified.

RESULTS AND DISCUSSIONS

The database screening has led to a collection of over sixty scientific publications, dated from 2007 to 2018. The authors have reported the use of different types of extracts (aqueous, alcoholic or PEG extracts), as well as the use essential oils of different plants in testing the antimicrobial activity. Different methods have been used to test the antimicrobial activity of different plants products. The most usual method is the agar diffusion method described by different authors or clinical standards in USA or Europe (Brown, 1978; Das et al., 2010). The findings are described in the following, grouped in annual or perennial plants, herbaceous or woody groups.

1) Annual / Biennial plants

The reported antimicrobial activity of annual/biennial plants from Romanian flora are synthetized in Table 1 and their appurtenance to botanical family is clearly specified.

There are different studies targeting the botanical family Lamiaceae, which includes most of the aromatic plants like *Origanum vulgare* L., *Melissa officinalis* L. or *Ocimum basilicum* L.; ethanolic extracts of aerial parts of these plants have been proven to have some antibacterial activity on *Listeria monocytogenes, Stahpylococcus aureus* (Benedec et al., 2015), as well as antifungal activity on *Candida albicans* (Tuchila et al., 2008; Benedec et al., 2015).

Table 1. Romanian annual herbaceous plants with antimicrobial activity

Plant species	Botanical family	Plant part	Antimicrobial activity		Reference
			Bacteria	Fungi	
Origanum vulgare L.	Lamiaceae	hb; fl; fs	*Listeria monocytogenes* *Stahpylococcus aureus* *E. coli* *Salmonella enteridis*	*Candida albicans*	Benedec et al., 2015 Dobre et al., 2011 Sandru et al., 2015
Melissa officinalis L.	Lamiaceae	hb; fl; fs	*Listeria monocytogenes* *Stahpylococcus aureus*	*Candida albicans*	Benedec et al., 2015 Hancianu et al., 2008
Ocimum basilicum L.	Lamiaceae	hb; fl; fs	*Listeria monocytogenes* *Staphylococcus aureus* *E. coli* *Streptococcus cricetus*	*Candida albicans*	Benedec et al., 2015 Stefan et al., 2011 Tuchila et al., 2008 Vlase et al., 2014
Thymus vulgaris L.	Lamiaceae	hb; fl; fs	*Staphylococcus aureus* *Klebsiella pneumoniae* *Salmonella typhimurium* *E. coli* *Enterococcus faecalis* *Salmonella enteridis* *Pseudomonas aeruginosa* *Listeria innocua* *Streptococcus pyogenes*	*Candida albicans* *Aspergillus niger*	Boruga et al., 2014. Dobre et al., 2011 Grigore Armatu et al., 2012 Varga et al., 2015
Thymus pulegioides	Lamiaceae	hb	*E. coli* *Enterobacter cloacae* *Proteus mirabilis* *Bacillus subtilis* *Micrococcus flavus* *Staphylococcus aureus* *Streptococcus faecalis* *Pseudomonas aeruginosa* *Listeria innocua* *Streptococcus pyogenes*	*Candida albicans*	Pavel et al., 2010 Varga et al., 2015
Thymus glabrescens	Lamiaceae	hb	*Salmonella typhimurium* *Pseudomonas aeruginosa* *Proteus mirabilis* *Listeria innocua* *Streptococcus pyogenes*	*Candida albicans*	Pavel et al., 2010 Varga et al., 2015
Satureja hortesis	Lamiaceae	hb	*Streptococcus cricetus* *Staphylococcus aureus*	*Botrityis cinerea* *Candida albicans*	Sesan et al., 2015 Tuchila et al., 2008
Anethum graveolens	Apiaceae	hb; fl; fs; sm	*Shigella flexneri* *Klebsiella pneumoniae* *Salmonella typhimurium* *E. coli*		Jianu et al., 2012
Tropaeolum majus	Tropaeolaceae	hb	*Pseudomonas aeruginosa* *Salmonella* sp. *Bacillus* sp.	*Candida albicans*	Butnariu et Bostan, 2011
Veronica officinalis	Plantaginaceae	hb	*Listeria monocytogenes* *Listeria ivanovii*	-	Mocan et al., 2015a Mocan et al., 2015b
Veronica teucrium	Plantaginaceae	hb	*Staphylococcus aureus* *Bacillus cereus* *Enterococcus faecalis* *Peptostreptococcus anaerobius*	-	Mocan et al., 2015a Mocan et al., 2015b
Veronica orchidea	Plantaginaceae	hb	*Listeria monocytogenes* *Listeria ivanovii* *Peptostreptococcus anaerobius*	-	Mocan et al., 2015a Mocan et al., 2015b
Veronica persica Poiret	Plantaginaceae	hb; fl; fs		*Aspergillus niger* *Penicillium hirsutum*	Fierascu et al., 2018
Arctium lappa	Asteraceae	rx fl	*E. coli* *Salmonella abony* *Staphylococcus aureus* *Staphylococcus epidermidis*	*Aspergillus niger* *Penicillium hirsutum*	Fierascu et al., 2018 Ionescu et al., 2013 Pirvu et al., 2017
Xanthium strumarium	Asteraceae	hb	-	*Phytophthora infestans*	Rodino et al., 2013
Cnicus benedictus	Asteraceae	fs	*Salmonella typhimurium* *Salmonella enteritidis*, *Shigella sonnei* *Staphylococcus aureus* *Streptococcus pyogenes* *Proteus vulgaris*, *E. coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*	-	Szabo et al., 2009
Calendula officinalis	Asteraceae	hb	*Klebsiella penumoniae* *S. aureus* *E. coli*	*Candida albicans*	Jianu et al, 2016
Tagetes patula	Asteraceae	fs		*Pythium* sp. *Botrytis cinerea*	Rodino et al., 2015 a Sesean et al., 2015

Legend: hb: herba (flowering aerial parts); fl: folium (leaves); fs: flos (flowers); nd: needles; fr: fructus (fruits); cx: cortex (bark); sm: semen (grains); rx: radix (roots); rh: rhizoma (rhizome); st: stipites (branches).

Similar results on essential oil of *Melissa officinalis* L. have been reported by Hancianu et al. (2008). Regarding *Origanum vulgare*, Sandru et al. (2015) have proven that essential oils made of the aerial parts have strong inhibitory effect on *E.coli*, same as other different species of *Ocimum* used as essential oils (Stefan et al., 2011). *Ocimum* sp. spectrum is completed by *Streptococcus cricetus* which is inhibited by the alcoholic extract (Tuchila et al., 2008).

In the same family, essential oils of *Thymus vulgaris* aerial parts, harvested in Mehedinţi County, have moderate to strong inhibition on *Staphylococcus aureus, Klebsiella pneumoniae*, *Salmonella typhimurium*, *E. coli, Enterococcus faecalis* and *Candida albicans* (Boruga et al, 2014); the authors correlate this activity with the presence of phenolic compounds (thymol) and terpene hydrocarbons (γ-terpinene). Some other authors reported higher antibacterial activity of thyme extracts originated in Southern Romania (Dobre et al., 2011), as well as anti-fungal effect (Grigore Armatu et al., 2012).

Other *Thymus* species have been investigated. Aerial parts of *Thymus pulegioides* collected at the flowering stage from two areas of the Bucegi Mountains at different altitudes (1000 and 1800 m above sea level) and aerial parts of *T. glabrescens* from the district of Gorj have been used for essential oils extraction (Pavel et al., 2010). *Escherichia coli*, *Enterobacter cloacae*, *Proteus mirabilis*, *Bacillus subtilis* and *Micrococcus flavus* were the strains most susceptible to *T. pulegioides* essential oil. *T.glabrescens* essential oil inhibited the growth of *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Proteus mirabilis*. The authors have related the inhibitory activity tothe presence of monoterpenoid alcohols in this sample, especially of geraniol (55.5%), which manifests an antiseptic activity comparable to that of thymol, often against *Pseudomonas*. All the tested samples showed antifungal effects by inhibiting the growth of *Candida albicans*.

The *Thymus* sp. spectrum of antibacterial activity is completed with data reported by Varga et al. (2015). Essential oils of four different *Thymus* species (*T. vulgaris, T. serpyllum, T. pulegioides,* and *T. glabrescens*) harvested in Mures county have been proven to inhibit the growth of *Pseudomonas aeruginosa*, *Listeria innocua* and *Streptococcus pyogenes*.

In the same family (Lamiaceae), *Satureja hortensis* harvested in Southern Romania has been reported to have antifungal effect on *Botrytis cinerea* (Sesan et al., 2015). Aqueous extracts of *Satureja hortensis* from Banat county have inhibited *Streptococcus cricetus*, while in alcoholic extract inhibited *Staphylococcus aureus* and *Candida albicans* (Tuchila et al., 2008).

The list of aromatic plants with antimicrobial activity is also completed by the dill. Essential oils from inflorescences, stems, immature and mature seeds of *Anethum graveolens* L. grown in Western Romania (Timis county) were isolated by steam distillation and tested on different bacteria. Significant antimicrobial activity was recorded against *Shigella flexneri, Klebsiella pneumoniae, Salmonella typhimurium* and *E. coli,* while no inhibitory effects were observed against *Streptococcus pyogenes* and *Staphylococcus aureus*, results which is partially in contradiction with other reported results (Jianu et al., 2012).

From a plant mainly cultivated as ornamental plant, *Tropaeolum majus*, Butanriu and Bistan (2011) have extracted essential oils starting from dehydrated leaves and flowers harvested in Timis county. The authors assumed that the antimicrobial action is determined by the phenols and metil-ethers identified in the *T. majus* extracts, but also by the tymol and carvacrol present in the volatile oil; the volatile oils tested presented a wide range of action over both Gram-positive and Gram–negative species. The most sensitive microorganism to the action of the tested natural compounds of *T. majus* proved to be *P. aeruginosa* and *C. albicans*, followed by *Salmonella* sp. and *Bacillus* sp, while the most resistant is the *E. coli* stem.

A plant considered annual, but sometime being an over winter specie, is *Veronica persica* Poiret. Crude hydroalcoholic extracts of its aerial parts originated in Pitesti hills showed important inhibitory effect on two pathogenic fungal species, *Aspergillus niger* and *Penicillium hirsutum* (Fierascu et al., 2018). This results have completed the image of antimicrobial effect of *Veronica* sp. described by Mocan et al. (2015), which have proven that

V. officinalis, V. teucrium and *V. orchidea* have inhibitory effect on *Staphylococcus aureus*, *Listeria monocytogenes* and Listeria *ivanovii*.
Other species of *Veronica* genus have been studied by Mocan et al. (2015 a, b). Hydroalcoholic extracts of aerial parts harvested in Cluj county shows that in the case of *V. officinalis*, the most sensitive bacterial strains were *Listeria monocytogenes* and *Listeria ivanovii*; regarding *V. teucrium* antibacterial activity, the strains of *Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus faecalis* have been the most sensitive. Referring to the *V. orchidea* extract, the most sensitive strains were *Listeria monocytogenes* and *Listeria ivanovii*. Also, the extracts of *V. teucrium* and *V. orchidea* have been proven to have antibacterial activity, on *Peptostreptococcus anaerobius*, an anaerobic Gram-positive bacteria responsible for clinical infections. The authors come with the assumption that the activity of *Veronica* ethanolic extracts against Gram-positive bacteria like *L. monocytogenes*, *L. ivanovii* and *S. aureus* could be attributed at least in part to their high β-sitosterol content but also to the presence of campesterol and stigmasterol and may be might be influenced also by the presence of hispidulin. Relatively recently, have been given special attention to the biennial *Arctium lappa*. Crude roots hydroalcoholic extract proved inhibitory effect on *Escherichia coli, Salmonella abony* (Ionescu et al., 2013), as well as on fungi as *Aspergillus niger* and *Penicillium hirsutum* (Fierascu et al., 2018). Authors have attributed the antimicrobial properties to the phenolic acids content (such as chlorogenic acid, rutin, quercitrin, luteolin, p-coumaric acid, caffeic acid and quercetin). In 2017, results obtained by Pirvu et al. suggest the potential uses of *Arctii folium* whole (70%, v/v) ethanol extract in restoring the activity of the antibiotics affected by microbial resistance, as well as inhibitory effect on *Stapyloccous epidermidis.*
A thistle-like plant from Asteraceae family, *Cnicus benedictus* in different extracts of immature capitulum harvested in North-Western Romania during prebloom period, have been proven to have a very large antibacterial spectrum, from *Salmonella typhimurium*, *Salmonella enteritidis* to *Shigella sonnei*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Proteus vulgaris*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Enterococcus faecalis* (Szabo et al., 2009).
From Asteraceae family have been taken into account also a species growing as weed, *Xanthium strumarium*. Ethanolic extracts of aerial parts of the plant have been demonstrated to have inhibitory effect on the growth of a phytopathogenic fungi, *Phytophthora infestans*, the causative agent of late blight in tomatoes and potatoes (Rodino et al., 2013). Further, Rodino et al. (2015 a) have tested another Asteraceae representative, *Tagetes patula* (marigold) on the phytopathogenic fungi *Pythium* sp., which can cause serious diseases such as damping off, seed rot, root rot and soft rot in wheat, maize, soybean, peppers, bean, cucumber, tomato. Ethanolic extracts of marigold flowers harvested from Southern Romanian from non-polluted sites, exhibited moderate to high inhibition on the fungal specie. Other authors (Sesan et al., 2015) reported *Tagetes* sp. extracts as having good antifungal activity on *Botrytis cinerea*.
Another annual member of Asteraceae family, *Calendula officinalis*, rarely studied for its antimicrobial activity, as essential oils of aerial parts has inhibited the growth of *Klebsiella penumoniae, S. aureus, E. coli* and of the fungus *Candida albicans* (Jianu et al., 2016).
There are some other annual plants studied for their antimicrobial activity, activity which have been proven to be weak on Romanian extracts, even some other reports are opposite. An example is *Agrimoniae herba* ethanolic extract which has only a weak inhibitory effect on *Pseudomonas aeuroginosa* (Pirvu et al., 2016).

2) Perennial plants

The reported antimicrobial activity of perrenial plants from Romanian flora are synthetized in Table 2 (herbaceous plant) and Table 3 (shrubs and trees), including their appurtenance to botanical family and the plants' part tested for the inhibitory activity.

Herbaceous plants

Different species from Asteraceae family have been proven to have inhibitory activity on pathogenic microorganisms. *Achillea* sp.

Table 2. Romanian perennial herbaceous plants with antimicrobial activity

Plant species	Botanical family	Plant part	Antimicrobial activity		Reference
			Bacteria	Fungi	
Achillea distans	Asteraceae	fs	*Listeria monocytogenes* *Stahpylococcus aureus*		Benedec et al., 2013
Achillea shurii	Asteraceae	fs	*Listeria monocytogenes* *Staphylococcus aureus* *Salmonella typhimurium*		Benedec et al., 2016
Achillea millefolium	Asteraceae	hb	*Klebsiella pneumoniae* *Salmonella typhimurium* *Staphylococcus aureus*	*Candida albicans*	Jianu et al., 2016
Achillea collina	Asteraceae	fs	*E. coli, Shigella flexneri* *Klebsiella pneumoniae* *Salmonella typhimurium* *Staphylococcus aureus*		Jianu et al., 2015
Artemisia spp.	Asteraceae	fs		*Sclerotinia sclerotiorum*	Badea and Delian, 2014
Tanacetum vulgare	Asteraceae	fs	*Bacillus cereus* *Staphylococcus aureus*		Muresan, 2015 Muresan et al., 2015
Inula helenium	Asteraceae	rx	*E. coli, Enterococcus faecalis* *Bacillus cereus* *Staphylococcus aureus*	*Candida albicans* *Candida parapsilosis*	Diguta et al., 2014
Santolina rosmarinifolia	Asteraceae	hb; fl; fs	*Staphylococcus aureus*	*Candida albicans*	Ioannou et al., 2007
Cynara scolymus	Asteraceae	fl	*E. coli, Salmonella abony* *Listeria innocua, Bacillus cereus*		Ionescu et al., 2013 Vamanu et al., 2011
Taraxacum officinale	Asteraceae	fl	*Escherichia coli* *Salmonella abony*		Ionescu et al., 2013
Eupatorium cannabium	Asteraceae	fl	*Escherichia coli* *Bacillus subtilis*	*Candida albicans*	Purcaru et al., 2015
Salvia officinalis	Lamiaceae	hb; fl; fs	*Listeria monocytogenes* *Stahpylococcus aureus* *Klebsiella pneumoniae*	*Candida albicans*	Benedec et al., 2015 Ilie et al., 2016
Rosmarinus officinalis	Lamiaceae	hb; fl; fs	*Listeria monocytogenes* *Stahpylococcus aureus*	*Candida albicans* *Aspergillus flavus* *Aspergillus ochraceus*	Benedec et al., 2015
Hyssopus officinalis	Lamiaceae	hb; fl; fs	*Listeria monocytogenes* *Stahpylococcus aureus* *P. aeruginosa*	*Candida albicans*	Benedec et al., 2015 Jianu et al., 2016 Mihai and Popa, 2015 Vlase et al., 2014
Mentha piperita	Lamiaceae	hb	-	*Candida albicans* *Botrytis cinerea*	Jianu et al., 2016 Sesan et al., 2015
Mentha smithiana	Lamiaceae	hb	-	*Candida albicans*	Jianu et al., 2016
Mentha spicata	Lamiaceae	hb	*Listeria monocytogenes*	*Candida albicans*	Moldovan et al., 2014
Mentha rotundifolia	Lamiaceae	hb	*Listeria monocytogenes*	*Candida albicans*	Moldovan et al., 2014
Ajuga genevensis	Lamiaceae	hb	*Staphylococcus aureus* *Pseudomonas aeruginosa* *Listeria monocytogenes,* *E. coli, Salmonella typhimurium*		Toiu et al., 2016
Teucrium chamaedrys	Lamiaceae	hb	*Staphylococcus aureus*	*Candida albicans*	Vlase et al., 2014
Hypericum perforatum	Hypericaceae	hb	*S. aureus, S. typhimurium* *E. coli*	*Candida albicans*	Jianu et al., 2016
Eryngium campestre	Apiaceae	hb; fl; fs	*Staphylococcus aureus* *Stahpylococcus epidermidis* *Pseudomonas aeruginosa*		Conea et al., 2016
Humulus lupulus	Cannabaceae	fs	*Bacillus subtilis*, *E. coli* *Enterococcus faecalis*, *Bacillus cereus*, *Staphylococcus aureus, Enterobacter cloacae,* *Pseudomonas fluorescens,*	-	Arsene et al., 2015
Hedera helix	Araliaceae	fs; fr	*Stahpylococcus aureus* *Listeria monocytogenes*		Pop et al., 2017
Allium ursinum *Allium sativum*	Amaryllidaceae		*Bacillus subtilis* *Staphylococcus aureus* *Streptococcus pyogenes* *E.coli*	*Aspergillus glaucus* *Geotrichum candidum* *Candida albicans* *Botrytis cinerea*	Lupoae et al., 2013 Sesan et al., 2015
Helianthemum nummularium	Cistaceae	hb	*E. coli, Staphylococcus aureus* *Salmonella typhimurium* *Salmonella enterritidis* *Pseudomonas aerugionsa*	*Candida albicans*	Pirvu et al., 2017a
Epilobium hirsutum	Onagraceae	hb	*Staphylococcus aureus* *E. coli*		Pirvu et al., 2014 Pirvu et al., 2015
Chelidonium majus	Papaveraceae	hb		*Botrytyis cinerea*	Parvu et al, 2011
Glycyrrhiza glabra	Fabaceae	rx		*Pythium* sp.	Rodino et al., 2015a
Paeonia officinalis	Paeoniaceae		*E. coli, Pseudomonas aeruginosa* *Salmonella abony*, *Staphylococcus aureus* *Enterococcus faecalis* *Brevibacterium flavum* *Sarcina* sp., *Bacillus cereus*	*Aspergillus niger*	Soare et al., 2012

Legend: hb: herba (flowering aerial parts); fl: folium (leaves); fs: flos (flowers); nd: needles; fr: fructus (fruits); cx: cortex (bark); sm: semen (grains); rx: radix (roots); rh: rhizoma (rhizome); st: stipites (branches).

Is one of the most studied in the family. *Achillea distans* Waldst. et Kit. ex Willd., found in the Rodna Mountains (a subdivision of the Eastern Carpathians in Northern Romania), is confirmed as a native species of the Romanian flora; its flowers hydroalcoholic extract showed inhibitory activity on Gram-positive bacteria as reported by Benedec et al (2013). From the same family, hydroalcoholic extract of *Achillea schurii* Sch.-Bip., an endemic species from Romania, has revealed a remarkable inhibitory effect on *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella typhimurium* (Benedec et al., 2016).
Essential oil of inflorescence harvested from *Achillea millefolium* and its hybrid *Achillea collina* Becker growing wild in Western Romania inhibited most strongly the growth of *E. coli*, followed by *Shigella flexneri, Klebsiella pneumoniae*, *Salmonella typhimurium* and *Staphylococcus aureus*. No effects were observed against *Clostridium perfringens* and *Streptococcus pyogenes* (Jianu et al., 2015; Jianu et al., 2016). The authors assumed this could be the results of the inhibitory effects exhibited by the major constituents of the analyzed essential oils, respectively chamazulene, caryophyllene and β-pinene; also they noticed the presence of certain minor components, known for their strong antimicrobial activity, such as limonene, *α*-pinene or 1.8-cineole.
Essentila oils obtained by hydro distillation, from *Artemisia* spp growth in different Romanian areas, as spontaneous flora or as cultivated species have been tested against fungal pathogen *Sclerotinia sclerotiorum* (Lib.) de Bary, from carrots roots stored in the refrigerator (Badea and Delian, 2014); minimum inhibitory concentration (MIC) was found to be 2400 µL L-1 for *A. santonica, A. pontica, A. annua, A. austriaca, A. dracunculus, A. lerchiana, A. vulgaris* and *A. vulgaris* var. *pilosa*.
Tanacetum vulgare is known mainly for its toxicity and insect repelent properties. Essential oils and ethanolic extracts of this plant, harvested in Transylvania (Sibiu and Alba county) exhibited moderate inhibition on *Staphylococcus aureus and Bacillus subtilis*, but low activity on *E. coli* and *Pseudomonas aeruginosa* (Muresan, 2015; Muresan et al., 2015).
The Asteraceae list is completed by *Inula helenium*; the ethanolic extracts were obtained from the roots of plants harvested in Brasov county (Transilvania); moderate to high bacterial inhibition have been shown on *Escherichia coli, Enterococcus faecalis, Bacillus cereus* and *Staphylococcus aureus*; meanwhile, moderate anti-*Candida* effects have been proven (Diguta et al., 2014).
Essential oils of the flower heads and leaves of *Santolina rosmarinifolia* L. were obtained through hydrodistillation and tested against Gram-positive and Gram-negative bacteria strains and the fungus *Candida albicans* (Ioannou et al., 2007). The highest inhibitory potential has been shown on *Staphylococcus aureus* and *Candida albicans.*
In the same family (Asteraceae) two other species, *Cynara scolymus* and *Taraxacum officinale* have proven antibacterial activity in hydroalcoholic leaves extracts against *Escherichia coli* and *Salmonella abony* (Ionescu et al., 2013). Meanwhile, Vamanu et al. (2011) has reported that freeze-dried ethanolic extracts of *Cynara scolymus* harvested in Hunedoara county (Transylvania) have significant inhibitory effect on *Listeria innocua and Bacillus cereus.*
Purcaru et al. (2015) have tested different dried leaves *Eupatorium cannabium* extracts made of a Romanian cultivar from Brasov county. In the case of the chloroformic extract and hydro-alcoholic extract the inhibitory activity has been noticed only in the case of *Escherichia coli* and *Bacillus cereus*, as well as on the dimorphic yeast *Candida albicans*. No clear inhibition has been noticed in the case of *Staphylococcus aureus, Enterococcus faecalis* and *Aspergillus niger*.
Lamiaceae family is on the top list of plants tested for their antimicrobial activity. Benedec et al. (2015) has proven that the rosmarinic acid from *Salvia officinalis* L. and *Rosmarinus officinalis* L. has strong antibacterial effect on Gram positive bacteria, even higher than gentamicin; similarly, strong effect has been noticed against *Candida albicans*, higher than fluconazole. Meanwhile, essential oils from aerial part of *Salvia officinalis* originated in Arad county showed strong inhibitory effect of

Staphyloccus aureus and *Klebsiella pneumoniae* (Ilie et al., 2016). The antimicrobial activity recorded have been attributed mainly to the major components of *S. officinalis* essential oils, i.e., camphor, alpha-thujone and alpha-humulene, recognized for their biological activities. Also, essential oils and terpens extracted from *Rosamarinus officinalis* have been proven to have antifugal effects, both on growth and sporulation of *Aspergillus flavus* and *Aspergillus ochraceus*; lower effect have been registered on *Aspergillus niger* (Mihai and Popa, 2015).

Remaining in the same Lamiaceae family, in Romania has been reported for the first time antimicrobial activity of essential oils of *Mentha smithiana* (Jianu et al., 2016). Aside *Mentha piperita,* their essential oils inhibited mainly the Gram-positive bacteria, as well as the fungus *Candida albicans.* Similar results have been obtained by the same authors in the case of essential oils of *Hypericum perforatum*. Also. *Mentha* sp. has been reported to have significant antifungal activity on *Botrytis cinerea* (Şesan et al., 2015). Other species of *Mentha* genus have been reported for anti-*Candida* activity by Moldovan et al. (2014), respectively extracts of *M. spicata* subsp. *crispata* and *M. x rotundifolia.* Same research group reported that other *Mentha* sp. extracts have strong inhibitory activity on *Listeria monocytogenes.*

Aerial part of *Ajuga genevensis* harvested from wild populations from Cluj county at full flowering stage, in alcoholic extracts showed high inhibitory activity against *Staphylococcus aureus*, followed by *Pseudomonas aeruginosa, Listeria monocytogenes, Escherichia coli* and *Salmonella typhimurium* (Toiu et al., 2016).

Lamiaceae family list of plants with animicorbial activity is completed by the ornamental *Teucrium chamaedrys*. Aerial parts harvested during summer in Sibiu county (Transilvania) have been prepared as ethanolic extract; the extract showed stronger antibacterial activity against *S. aureus* than gentamicin used as reference antibiotic, as well as antifungal activity against *Candida albicans,* higher than fluconazole(Vlase et al., 2014).

From Apiaceae family, different species of *Erymgium* have been tested for their antimicrobial activity. Tincture of aerial plants from *E. planum* and *E. campestre* from Cluj county and *E. maritimum* from Constanta county have proven to have moderate antibacterial activity on *Stahpylococcus aureus* and *Stahpylococcus epidermidis* and high inhibitory activity on *Pseudomonas aeruginosa*, especially in the case of *E. campestre* (Conea et al., 2016); authors assumed that the activity of *Eryngium* tinctures probably results from the synergistic effect of triterpene saponins, polyphenols, sterols, pectin and other active compounds.

Humulus lupulus (common hop) from Southern Romania, an herbaceous climbing plant, as hydroalcoholic extracts of female inflorescences, has been proven to have antagonistic effect on both Gram-positive and negative bacteria (Aresene et al., 2015). In the case of hope the substances associated with antibacterial activity are humulone, lupulone and xanthohumol (Cermak et al., 2017).

Another climbing plant, *Hedera helix* harvested in Cluj county as leaves, flower and immature fruits has been tested by Pop et al. (2017). They have arrived to the conclusion that the immature fruits extract showed a significant activityagainst *S. aureus*, followed by the flower extract with a good growth inhibitory effect against the same bacterial strain. Both immature fruits and flowers extracts possess appropriate antibacterialcapacity against *L. monocytogenes*.

Among perennial bulbous of Romanian wild flora has been tested *Allium ursinum* in hydroalcoholic or acetic acid extracts obtained from different parts (leaves, roots, bulbs). The extracts inhibited the growth of different altering or pathogen fungi like *Aspergillus glaucus*, *Geotrichum candidum* and *Candida albicans*, as well as on different Gram-positive and Gram-negative bacteria (Lupoae et al., 2013). The authors recommend their use in the food industry as additive. Şesan et al. (2015) have also demonstrated that *Allium sativum* extracts have inhibitory effects on *Botrytis cinerea* (grey mould)affecting cultures of *Ribes nigrum*.

The hydroalcoholic extract of *Chelidonium majus* (Papaveraceae) obtained from powder of dried aerial plant organs collected from a private homegarden in Cluj county had antifungal effect against *B. cinerea* (Pârvu et al., 2011). Another phytopathogenic fungi, *Pythium* sp., have been proven to be inhibited by ethanolic extracts of *Glycyrrhiza glabra* (Fabaceae) roots harvested in Southern Romania from non-polluted sites (Rodino et al., 2015a).

A novelty may be considered the studies conducted by Pîrvu et al. (2017 a) regarding the antimicrobial activities of extracts from rock rose (*Helianthemum nummularium* Mill.) harvested in July from Romanian Carpathian Mountains. These extracts show certain antimicrobial activity on *E. coli*, as well as weak to moderate activity on *S. aureus, S. typhimurium* and *S. enterritidis*; the list is completed by *Pseudomonas aueriginosa* and *Candida albicans.*

Among perennial herbaceous plants tested for antimicrobial activity an ornamental plant was in the research attention. Red petals of *Paeonia officinalis*, in ethanolic and methanolic extracts have shown strong inhibitory activity on bacteria and fungi, respectively *Escherichia coli, Pseudomonas aeruginosa, Salmonella abony*, *Staphylococcus aureus, Enterococcus faecalis, Brevibacterium flavum, Sarcina* sp.,

Table 3. Romanian perennial woody plants with antimicrobial activity

Plant species	Botanical family	Plant part	Antimicrobial activity		Reference
			Bacteria	Fungi	
BUSHES/SHRUBS					
Lavandula angustifolia *Lavandula x intermedia*	Lamiaceae	fs	*Shigella flexneri* *Staphylococcus aureus* *E. coli*	*Candida albicans*	Jianu et al., 2013 Robu et al., 2016
Viburnum opulus	Caprifoliaceae	hb; fl; fs	*Staphylococcus aureus* *Staphylococcus epidermidis*		Bubulica et al., 2012
Lonicera tatarica	Caprifoliaceae	hb; fl; fs	*Staphylococcus aureus* *Staphylococcus epidermidis*		Bubulica et al., 2012
Aronia melanocarpa	Rosaceae	fr; fl	*Vibrio vulnificus,* *V. cholera, V. mimicus* *E. coli* *Enterococcus faecalis*		Giupana et al., 2016
Lycium barbarum	Solanaceae	fl; fs	*Staphylococcus aureus* *Listeris monocytogenes* *Bacillus subtilis*		Mocanu et al., 2014 Mocanu et al., 2015c
Lycium chinense	Solanaceae	fl	*Staphylococcus aureus* *Bacillus subtilis* *Listeria monocytogenes* *Salmonella thyphimurium*		Mocanu et al., 2014
Sambucus ebulus	Adoxaceae	fr	*Pseudomonas fluorescens* *Enterococcus faecalis*		Rodino et al., 2015b
TREE					
Juniperus communis	Cupressaceae	fr	*Bacillus subtilis* *Streptococcus luteus* *Staphylococcus aureus* *Escherichia coli*		Ivopol et al., 2016
Abies alba	Pinaceae	nd; cx	*Bacillus subtilis* *Streptococcus luteus* *Staphylococcus aureus* *Escherichia coli*		Ivopol et al., 2016 Sandru et al., 2015
Picea abies	Pinaceae	nd	*Staphylococcus aureus* *Bacillus cereus* *Proteus vulgaris*	*Candida albicans* *Aspergillus niger*	Radulescu et al., 2011
Pinus sylvestris	Pinaceae	nd; cx	*Bacillus subtilis* *Streptococcus luteus* *Staphylococcus aureus* *Escherichia coli*		Ivopol et al., 2016
Pinus cembra L.	Pinaceae	nd; cx	*Staphylococcus aureus* *Sarcina lutea* *Bacillus cereus* *Escherichia coli* *Pseudomonas aeruginosa*	*Candida albicans*	Apetrei et al., 2011 Apetrei et al., 2013
Fagus sylvatica	Fagaceae	fl	*Staphylococcus aureus*		Pirvu et al., 2014
Robinia pseudoacacia	Fabaceae	fs; sm cx; fl	*Staphyloccous* sp., *Streptococcus* sp. *E coli., Pseudomonas aeruginosa,* *Proteus* sp., *Salmonella enterica*	*Candida albicans*	Rosu et al., 2012
Cydonia oblonga	Rosaceae	fl	*Staphylococcus aureus* *Escherichia coli* *Pseudomonas aeruginosa*		Cerempei et al., 2016

Legend: hb: herba (flowering aerial parts); fl: folium (leaves); fs: flos (flowers); nd: needles; fr: fructus (fruits); cx: cortex (bark); sm: semen (grains); rx: radix (roots); rh: rhizoma (rhizome); st: stipites (branches).

Bacillus cereus and *Aspergillus niger* (Soare et al., 2012).

Bushes/Shrubs

Lavender (*L. angustifolia* Miller) and lavandin (*Lavandula x intermedia*) are well known for their medical and cosmetics applications. Essential oils obtained by steam distillation from fresh inflorescences harvested in Western Romania showed antimicrobial activity against *Shigella flexneri*, *Staphylococcus aureus*, *E. coli* and *Salmonella typhimurium*, while *Streptococcus pyogenes* was not sensitive to their action (Jianu et al., 2013). The authors emphasize the fact that even in the absence of active principles like linalool and linalyl acetate, considered responsible for the antibacterial and antifungal properties of essential oils obtained from different species of *Lavandula.* This results looks to be in contrast with results reported by Robu et al. (2016) on essential oils of lavandin (*Lavandula hybrida* Reverchon) harvested in North-Eastern Romania (Neamt county); these oils showed no activity against Gram-negative strains; also the results showed that the antistaphylococcal activity is reduced, while there is a moderate antifungal activity.

Two bushes belonging to Caprifoliaceae family, *Viburnum opulus* and *Lonicera tatarica* from Craiova, Dolj county have been tested by Bubulica et al. (2012); aqueous extracts of aerial parts (stem, flower buds, fruit pulp) have been tested on *Staphylococcus aureus* and *Staphylococcus epidermidis*. The results showed a higher inhibition in the case of *Lonicera tatarica* extracts.

Studies on bacterial strains isolated from wild birds captured in Danube Delta Biosphere Reservation proved that extracts of fresh fruits of *Aronia melanocarpa* has important inhibition on *Vibrio* spp. (*V. vulnificus, V. cholera, V. mimicus*), *E. coli, Enterococcus faecalis* (Giupana et al., 2016).

Ethanolic extracts of *Lycium* sp. (Solanaceae) leaves originated in Cluj county, have been tested for antimicrobial activity (Mocan et al., 2014). The authors reported that *L. chinense* extract was more active than *L. barbarum* against both Gram-positive and Gram-negative bacterial strains and that these species as important sources of flavonoids and chlorogenic acid. The best antibacterial activity was shown by *L. chinense* extract against *Bacillus subtilis*. Meanwhile, extract made of *L. barbarum* flowers was found to be more active on the Gram-positive bacterial strains; the best antibacterial activity was shown against *Staphylococcus aureus* (Mocan et al., 2015 c).

The perennial herbaceous extracts of *Epilobium hirsutum* harvested in Prahova county inhibited both *Stapyloccous aureus* and *E. coli* (Pirvu et al., 2014). Same group (Pîrvu et al., 2015) suggested an augmented antimicrobial potency on *Stapyloccous aureus* of the combination kaempferol-caffeic acid derivates (aqueous fraction) than myricetin-gallic acid derivate (ethyl acetic fraction).

The dwarf elderberry (*Sambucus ebulus*) used in traditional medicine, has proven to have antibacterial effects on *Pseudomonas fluorescens* and *Enterococcus faecalis* when used as ethanolic extract made of fruits (Rodino et al., 2015 b).

Trees

Conifers are widely used for the extraction of essential oils and their volatile oils contain mainly monoterpene (Ivopol et al., 2016). Among the conifers *Pinus* sp. (Pinaceae) has been widely studied. *Pinus cembra* L. from Carpahtian Mountains, bark and needles, have antimicrobial effects against *Staphylococcus aureus*, *Sarcina lutea*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* (Apetrei et al., 2011; Apetrei et al., 2013). Common *Pinus sylvestris* essential oils from needles and sprouts showed inhibitory effects on *Bacillus subtilis, Streptococcus luteus, Staphylococcus aureus* and *Escherichia coli*; similar results have been obtained by the use of essential oils from *Juniperus communis* berries and *Abies alba* needles and sprouts (Ivopol et al., 2016). Sandru et al. (2015) also proved inhibition on *E. coli* by the use of *Abies alba* essential oils.

Antimicrobial properties of volatile oil is olated from sprouts of *Picea abies* growing wild in Romanian Carpathian Mountains (Prahova Valley) have been tested by Radulescu et al. (2011). The most evident inhibitory effect was noticed against the Gram-positive (*Staphylococcus aureus*, *Bacillus cereus*), Gram-negative (*Proteus vulgaris*) and fungal strains (*Candida albicans*, *Aspergillus niger*).

There are authors which have tested more species from a specific Romanian region for their antimicrobial activity. For example, Pirvu et al. (2014) have focused on herbaceous and woody plants from Prahova county in propylene glycol solutions or in separate aqueous, ethyl acetate and chloroform fractions. Among the trees, extracts from leaves of *Fagus sylvatica* exhibited moderate inhibitory effect on *Stapyloccous aureus*, ethanolic extracts from flowers and seeds of *Robinia pseudoacacia* have inhibitory activity mainly on Gram-positive coci (*Staphyloccous* sp., *Streptococcus* sp.), while same extracts from bark and leafs inhibited *E coli, Pseudomonas aeruginosa, Proteus* sp., *Salmonella enterica* and *Candida albicans*.

As a novelty can be mentioned the use of fall quince (*Cydonia oblonga*) leavesoriginated in North-Eastern Romania for the production of natural dye; it has been proven (Cerempei et al., 2016) that such dye with mordant (silver nitrate) have a good antibacterial activity against Gram-positive (*S. aureus*) and Gram-negative (*E. coli* and *Ps. aeruginosa*); the authors assumed that a possible explanation can be that the wool-Ag-flavonoid complex has a larger surface area that gives antibacterial effect.

CONCLUSIONS

In our tentative to find out the interest of Romanian researchers to prove the antimicrobial activity of autochthonous flora from different regions and counties in our country, we have identified over sixty articles published in the time frame 2007-2018. We are aware that some other authors may have published in the subject and have escaped to our search.

The tested plants have been harvested from different geographical regions of Romania, from fields, hills and mountains; we have noticed more abundant information coming from Transylvania and Banat region, followed by Southern counties and Moldavia. In our search we have identified a total of 64 species from autochthonous flora taken into account for studies on antimicrobial activity, belonging to 21 botanical families. Among these species, 28.1% are annual species, 46.9% are herbaceous perennial and the rest (25%) are woody perennial species (bushes and trees). The antimicrobial studies have been mainly focused on species belonging to two botanical families, Asteracea and Lamiaceae, which represents 50% of the total studies species.

In terms of microbial species can be noticed an intensive focus on pathogenic Gram-positive and Gram-negative bacteria, responsible for clinical infections or food contamination. For 89% of the species have been reported antibacterial activity, while only 57.8% of the species have proven antifungal activity. The most reported susceptible fungus was *Candida albicans*; few reports are focused on filamentous fungi like *Aspergillus* sp., *Penicillium* sp, *Botrytis cinerea* or *Pythium* sp.

It has been noticed that some of the reports are novelty in the subject and the researchers have approached some spontaneous species little or not ever reported in the international databases (*e.g. Helianthemum nummularium, Cydonia oblonga, Paeonia officinalis*). This trend may be a solution for further research in the topic, as well as enlarging the studies on filamentous fungi, even if they are of medical or feed/food interest.

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