INHIBITORY EFFECTS OF MEDICAL PLANTS ON THE Candida albicans AND BACTERIAL GROWTH IN THE ORAL CAVITY

ABSTRACT: In this mini-review, the authors discuss the effects of ethanol extracts, essential oils and cytotoxicity of some medicinal plants and their compounds used in ethnomedicine in different geographic regions worldwide, including Serbia, on the growth, multiplication and pathogenicity of Candida albicans and bacteria that play the main role in the balance of the oral ecosystem. Various medicinal plants, such as Rosmarinus officinalis (Fam. Lamiaceae), Artemisia dracunculus, Artemisia absinthium (Fam. Asteraceae), exist in different geographic regions and continents, as well as in the Balkan region, and among them there are some indigenous species like Hypericum perforatum L. (Fam. Hypericaceae), Urtica dioica L. (U. dioica) (Fam. Urticaceae), Achillea millefolium L. (Fam. Asteraceae), Matricaria chamomilla L. (Fam. Asteraceae), Sambucus nigra L. (Fam. Caprifoliaceae), and Thymus serpyllum L. (Fam. Lamiaceae) with impressive antimicrobial activity against microorganisms originating from the oral cavity.

KEYWORDS: medicinal plants, Candida albicans, oral cavity microbiota, bacteria

INTRODUCTION

Medicinal plants had been used in traditional medicine as a part of national culture and traditional heritage long before the knowledge of chemical properties of bioactive substances and procedures of classical medicine were
widely distributed and accepted. In Russia, the first public pharmacy was opened in 1672 in Moscow, and in the same year, a herbalist manuscript entitled “Cool vineyard” was published (Shikov et al., 2014). The illustrative examples can be found in Russian phytotherapy, which has accumulated and adopted approaches that originated from European and Asian traditional medicine, due to its specific geographic location (Shikov et al., 2014). Besides geographic location, age, gender, farming, and stockbreeding are associated with the knowledge of plant resources. Historical, cultural and socioeconomic traits are essential to understand the local knowledge of useful plant diversity in a particular study area (Monroy-Ortiz et al., 2004). In many cases, the cultural factor means that the use of a species is more widespread than its ecological distribution. This may also explain the presence of synonyms and polysemies, which are useful for discussing ethnopharmacological data (Akerreta et al., 2007). The correlation between medicinal flora and floristic environment means that adaptation to the local area, even at small spatial scale, is in contrast to the effects of shared ancestry (Saslis-Lagoudakis et al., 2015).

Unfortunately, there are some mainly negative influences on the growth of medicinal plants: wild plant collection, residential and commercial development, livestock farming, ecosystem modifications, annual and perennial non-timber crops, wood, and pulp plantations, problematic native species/diseases, etc. Further, climate change and severe weather: droughts, fire and fire suppression, energy production and mining, logging and wood harvesting, dams and water abstraction, invasive non-native/alien species diseases, pollution, transport and infrastructure also should not be neglected (Allen et al., 2014).

Medicinal use of plants is common to all cultures, and it is usually part of the national heritage. There are ancient manuscripts like the Egyptian Ebers Papyrus (1500 BC) and Wu Shi Er Bing Fang (“Recipes for Fifty-Two Ailments”), dating from the 2nd century BC, discovered in 1973 in a tomb at Ma Huang Dui in Hunan Province, China. The Atharva Veda (India) dated back to 1200 BC. Egyptian priest doctors influenced the Greek medicine e.g. Hippocrates. Pedanius Dioscorides, a Greek physician, compiled ancient and contemporary herbal knowledge in his book *De Materia Medica* (*Peri hules iatrikēs*), that for more than thirteen centuries remained one of the principle medical textbooks throughout the civilized world. Claudius Galen, also known as Galen of Pergamon, was among the first who systematized the knowledge in the field of medicinal plants. Another mixing of cultures between European Crusaders and their Arab adversaries brought to Europe the knowledge of ancient Greek and Persian medicine (European Herbal & Traditional Medicine Practitioners Association. Herbal and Traditional Medicine).

In Serbia, the *Hilandar Medical Codex* is the most significant and best-preserved medieval Serbian manuscript containing documents on European medical science from the 12th to 15th century. Out of 167 recorded substances, 135 are of plant origin, 13 of animal origin, and 19 are of inorganic origin. The recorded plant species are categorized into 63 families, of which the most frequent are: Apiaceae, Lamiaceae, Asteraceae, Rosaceae and Fabaceae (Jarić et al., 2011).
European medicinal plants have been well described and its antibacterial action is very popular. *Dianthus superbus* (Fam. Caryophyllaceae) has long been used for the treatment of bacterial infections. The balsamic sage, *Salvia tomentosa* (LC) (Fam. Lamiaceae), is found to be an antibactericidal agent. Sore throat, cough, and colds have been treated by sage (*Salvia officinalis*). *Gypsophila perfoliata* (Fam. Caryophyllaceae) powder, derived from the root is used for wound healing. Bactericidal effect has formulations from the stems, flowers and fruits (Allen *et al.*, 2014). *Echinacea* (Echinacea spp., Fam. Asteraceae) is useful in relieving colds and upper respiratory infections (approved in Europe for these uses). Cranberry (*Vaccinium macrocarpon*, Fam. Ericaceae) is applied for reducing the risk of bladder infection. *Calendula* (Calendula officinalis, Fam. Asteraceae) has long been used to relieve inflammation of the mouth, throat, and stomach; it is popular as a topical cream or ointment to relieve rashes and irritation and to help heal wounds. Elderberry (*Sambucus nigra, S. canadensis*, Fam. Capryofoliaceae) flowers have been valued as a remedy for colds and fever for centuries. Garlic (*Allium sativum*, Fam. Amaryllidaceae) is a potent antimicrobial; it is often used to combat colds, ease sinus congestion, and stave off traveler’s diarrhea. Hibiscus (Fam. Malvaceae, *Hibiscus sabdariffa*) is used traditionally to ease sore throats and colds. Lemon balm (*Melissa officinalis*, Fam. Lamiaceae) is applied in topical creams used for fever blisters. Licorice (*Glycyrrhiza glabra*, Fam. Fabaceae,) is excellent for anti-inflammatory remedies, for smoothing mucous membranes, and for healing sore throats and coughs. *Thymus vulgaris*, (Fam. Lamiaceae) has application in relieving coughs, colds, and congestion. (Pizzorno and Murray, 2013). Its effect is so highly valued in general population that its Serbian name is Mother’s Sweetheart (in Serbian: majčina dušica).

In Serbia, in the region of Mt. Kopaonik, according to Jarić *et al.* (2007) the village people do not visit doctors except for surgical problems, and they use plants as their parents and grandparents did. Among wild plants most commonly used for medicinal purposes are: *Hypericum perforatum* L. (Fam. Hypericaceae), *Urtica dioica* L. (Fam. Urticaceae), *Achillea millefolium* L. (A. millefolium) (Fam. Asteraceae), *Matricaria chamomilla* L. (Fam. Asteraceae), *Sambucus nigra* L., and *Thymus serpyllum* L. (Fam. Lamiaceae). These plants were particularly highly recommended by the majority of the interviewed. The most frequently reported medicinal uses were for treating gastrointestinal ailments (50%), skin injuries and problems (25.6%), followed by respiratory, urinary-genital and cardiovascular problems (20.5%, 20.5%, 19.2%, respectively) (Jarić *et al.*, 2007). A slightly different situation is in Mt. Rtanj, in Eastern Serbia, where, because of depopulation, mainly elderly people were interviewed. The most common in traditional usage were the species of Labiatae (22%), Rosaceae (20%), and Compositae (13%). *H. perforatum* was the most popular medicinal plant. The most frequently reported medicinal uses of herbal drugs included remedies for the immune system (22.97%), respiratory system (15.77%), and digestive system disorders (15.32%) (Zlatković *et al.*, 2014).
Nowadays, in particular, there is a growing interest in herbs and their compounds applicable in contemporary remedies of conventional medicine based on scientific information and data (Clark, 1996; Wurglics et al., 2001; Rates, 2001; Salem and Werbovetz, 2006; Veeresham, 2012).

_Candida albicans_, MICROBIOTA OF ORAL CAVITY

Bacteria, mainly aerobic and microaerophilic streptococci and some yeasts such as _Candida albicans_ (C. albicans) populate the healthy human oral cavity. Their overgrowth can cause diseases known as candidiasis, stomatitis, gingivitis, or parodontopathy (Lamont et al., 2014). Furthermore, changes of pH, a balance between microorganisms, and the presence of specific substrate could enable and enhance the adherence ability of microorganisms involved in dental diseases (Glee et al., 1995). There is an opinion that _Candida_ overgrowth and adherence to structures in the oral cavity is of paramount importance for the development of an infection because its presence may change the stability of the sensitive ecosystem of the oral microflora (Glee et al., 1995).

_Candida albicans_ belongs to the Kingdom of Fungi, Division of Ascomycota, Class Saccharomycetes, Order Saccharomyceetales, Family Saccharomycetaceae and Genus: Candida (Lachance et al., 2011). Both yeast and hyphal forms (dimorphic) are present in infected tissue. Virulence properties of _C. albicans_ are the consequence of the dimorphic transition. Various sources of carbon and nitrogen possibly accelerate the growth of _C. albicans_ over other microbiota. The absence of glucose contributes to the very poor growth conditions for _C. albicans_ in the oral cavity (Cannon and Chaffin, 2001; Jenkinson and Douglas, 2002).

Infections caused by _Candida_ may be superficial or systemic. Superficial infections of the mucocutaneous tissues may include oropharyngeal candidiasis, involving the palate, tongue, and buccal mucosa (Jenkinson and Douglas, 2002; Alt-Epping et al., 2012; Stoopler and Sollecito, 2014). Generally, the risk of _Candida_ infection or colonization may be the consequence of the numerous predisposing factors. These factors are poor oral hygiene, cellular immune deficiencies, immunosuppression, inefficiency of the thymus, infection with human immunodeficiency virus (HIV), nutritional deficiencies, long-term use of antibiotic/radiation therapy, dental prostheses, _diabetes mellitus_, high carbohydrate diet, or heavy cigarette smoking (Jenkinson and Douglas, 2002; Wu et al., 2013). Regardless of aging, denture wearing constitutes a stable factor that has a positive influence on _Candida_ growth (Mizugai et al., 2007).

Successful survival of _C. albicans_ requires good adaptation, which includes adherence to the oral cavity structures, multiplication and invasiveness provided by degradative enzyme synthesis and secretion (Cannon and Chaffin, 2001). _C. albicans_ expresses adhesions rather in the mycelial form than in the form of the yeast (Fukazawa and Kagaya, 1997). In the ability of _C. albicans_ to adhere to the surfaces, cell superficies hydrophobicity has paramount importance, since hydrophobic cells adhere better to host tissue compared to hydrophilic cells.
(Glee et al., 1995). The abundance of phosphodiester-linked, acid-labile mannosyl groups may be associated with hydrophobicity (Masuoka and Hazen, 1997). During Candida life cycle, the presence of hydrophobic proteins on surface remains constant, however, hydrophilic proteins vary during growth (Hazen and Hazen, 1993).

C. albicans secretes a range of degradative enzymes such as aspartyl proteinases, lipases and hexosaminidase that may damage host tissues and contribute to C. albicans pathogenicity (Cannon and Chaffin, 1999). Adhesion, invasion and damage of the oral cavity by C. albicans depend not only on fungal morphology and activity, but also on the epithelial cell type and the differentiation stage of the epithelial cells. Data indicate that C. albicans can invade oral epithelial cells by inducing endocytosis and/or by active penetration (Dalle et al., 2010). Hyphal formation of C. albicans facilitates epithelial invasion via two routes: active penetration and induced endocytosis. While induced endocytosis is predominantly mediated by adhesin and invasin (als adhesin Als3), active penetration seems to be supported by hydrolase activity and mechanical pressure. Besides adhesion and morphogenesis, biofilm formation and morphological changes of multicellular structures are also the virulence factors of C. albicans. Candida utilizes the Ras/cyclic AMP (cAMP)/protein kinase A (PKA) signal transduction pathway to regulate many of adaptation mechanisms (Inglis and Sherloc, 2013).

ORAL HYGIENE

Oral hygiene, among other factors, depends on ingredients used in modern toothpaste formulations, which include abrasive agents, surfactants, humectants, thickening agents, flavoring, coloring agents, and antimicrobial agents. These antimicrobial agents include metal salts, phenols, herbal extracts, enzymes, essential oils, and bisbiguanides (Stamm, 2007; Yiğit et al., 2008). Toothpaste has been formulated to contain chemotherapeutic agents to improve the oral health, to produce inhibitory action on plaque formation as well as bacteria and Candida colonization (Gardiner et al., 2008; Yiğit et al., 2008; Sadeghi and Assar, 2009; Adwan et al., 2012). However, there is a different approach to the oral hygiene. Medicinal plants are the basic ingredients of toothpaste on the one hand, and on the other, the presence of herbal components is avoided.

Many investigations support some aspects of the use of traditional approach to the oral health. Traditional medicine can treat various infectious and chronic conditions. A research has shown that all kinds of chewing sticks described in ancient Ayurveda texts have medicinal and anti-cariogenic properties. Its oil pulling practice is claimed to cure about 30 systemic diseases. Amla (Emblica myrobalan, Fam. Phyllanthaceae) is a general rebuilder of the oral health. Bilberry fruit (Vaccinium myrtillus) and hawthorn berry (Crateagus oxycanthus, Fam. Rosaeae) stabilize collagen, strengthening the gum tissue. Liquorice root (Glycyrrhiza glabra) promotes anti-cavity action, reduces plaque, and has an antibacterial effect (Singh and Purohit, 2011). In Africa, several medicinal plants
are used for maintaining the oral hygiene: *Antidesma venosum*, Tul. (Fam. Phyllanthaceae), *Casearia barteri*, Mast (Fam. Flacourtiaiceae), *Citrus aurantifolia* Swing (Fam. Rutaceae), *Diospirus elliott* F. Whit, (Fam. Ebenaceae), *Garcinia kola* Heckel (Fam. Clusiaeae), *Jatropha curcas* Linn. (Fam. Euphorbiaceae), *Lecaniodiscus cupaniodes* (Fam. Sapindaceae), *Ocimum gratissimum* Linn. (Fam. Lamiaceae), *Vernonia amygdalina* Del. (Fam. Asteraceae), *Zanthoxylum zanthoxiloides* Waterm (Fam. Rutaceae), *Massularia acuminata* (G. Don.) Bull Ex Hoyle (Fam. Rubiaceae), and *Terminalia glaucescens* Planch. (Fam. Combretaceae) (Elujoba et al., 2005).

**ANTIFUNGAL ACTIVITY OF PLANTS AND ALGAE AGAINST C. albicans**

Numerous contemporary investigations support the common knowledge that some plants are used as traditional remedies against microorganisms including *C. albicans*. Since there has been a limited range of available antifungal drugs, especially those for resistant *C. albicans* strains, the prospect of preventing its colonization is becoming increasingly attractive. There are several approaches for preventing colonization by inhibiting *C. albicans* adherence: immunization, physical interference, salivary IgA, mucosal immune response, and application of soluble ligands (Cannon and Chaffin, 2001). However, there are not promising and cost-effective results. Perhaps, the use of traditional medicinal plants may take part in efficient treatment of oral candidiasis or as ingredients in toothpaste for prevention. Yet, there are not many reports related to Serbian autochthonous medicinal plants used in traditional medicine against *C. albicans* in the oral cavity (Tadić et al., 2008; Savikinet et al., 2009). Hawthorn [*Crataegus monogyna* Jacq. and *Crataegus oxyacantha* L.; sin. *Crataegus laevigata* (Poiret) DC., Fam. Rosaceae] berries ethanol extract had no effect on *C. albicans* (Tadić et al., 2008). Ličina et al. (2013) reported that ethanol, acetone, ethyl acetate and diethyl ether extract of *Origanum vulgare* L. (Fam. Lamiaceae) inhibits the growth of *C. albicans* at concentrations between 2.5 mg/mL and 10 mg/mL.

In one study conducted in Malaysia, *Brucea javanica* (Fam. Simaroubaceae) and *Piper betle* (Fam. Piperaceae) aqueous extracts had effects on the non-specific and specific adherence mechanisms of oral *C. albicans* by modifying the surface hydrophobicity of a cell wall and the characteristics of the experimental pellicle. Namely, exposing to pellicle treated with *P. betle* drastically reduced the adherence of *Candida tropicalis*, *C. albicans*, and *Candida krusei* by 86.01%, 61.41%, and 56.34%, respectively. *B. javanica* exhibited similar effects on *C. tropicalis* (89.86%), *Candida lusitaniae* (88.95%), *C. albicans* (79.74%), *Candida glabrata* (76.85%), and *C. krusei* (67.61%) (Nordin et al., 2013). Also, tincture from *Schinus terebinthifolius* (Fam. Anacardiacae) (Brazilian pepper tree) showed anti-fungal activity against *C. albicans*, probably by inhibition of the fungal cell wall formation (Alves et al., 2013). In Nairobi, Kenya, herbal extracts of *Leonotis nepetifolia* (Fam. Lamiaceae), *Biden pilosa* (Fam. Asteraceae), *Senna didymobotrya* (Fam. Fabaceae), *Toddalia asiatica*
(Fam. Rutaceae), and Physalis peruviana (Fam. Solanaceae) have a potential to control the multiplication of C. albicans (Maobe et al., 2013). One study inspired by traditional Indian medicine or Ayurveda showed that the ethanol extract of ginger powder has pronounced inhibitory activities against C. albicans. Ethanol itself has antifungal activity; ethanol extract of ginger had a synergistic activity (Supreetha et al., 2011). Further, there is an observation coming from India (Rajah Muthiah Medical College and Hospital, Annamalai University) that an expressed antifungal activity is observed for some plants: Syzygium jambolanum (Fam. Myrtaceae), Cassia siamea (Fam. Fabaceae), Caulerpa scalpelliformis (Fam. Caulerpaceae), and algae: green alga consisting of one cell and many nuclei, and Sargassum wightii (Fam. Sargassaceae), brown macroalga (Prabhakar et al., 2008).

ESSENTIAL OILS

Essential oils of thyme (T. vulgaris L.) and tea tree oil (Melaleuca alternifolia L.) (Fam. Myrtaceae) both caused changes in cell and colony morphology, but also in the metabolism of C. albicans (Rajkowska et al., 2014; Yigit et al., 2009). In addition, some data indicate that the essential oil of Rosmarinus officinalis (Fam. Lamiaceae) modulates C. albicans pathogenicity through its primary virulence factor, which is the suppression of germ tube formation (Gauch et al., 2014).

Investigation of the chemical composition and the activity against C. albicans of volatile oils obtained from herbs of the family Asteraceae, consisting of about 500 species, points out that many of its members possess the antifungal activity. Traditionally, the subgeneric taxonomy species Artemisia (Fam. Asteraceae) follows a system established by Besser (1829), who separated sections based on various combinations of disc and ray flower occurrences and fertility. Subsequent works of Rydberg (1916), who elevated the sections to the level of subgenera and created subordinate sections including section Tridentatae for the North American members of subgenus Seriphidium, modified Besser’s four sections (Abrotanum, Absinthium, Dracunculus, and Seriphidium). Current consensus is the recognition of three subgenera: Artemisia L. (5 Bessers’s Abrotanum 1 Absinthium), Dracunculus (Besser) Rydb., and Seriphidium (Besser) Rouy. However, based on karyotypic, chemotaxonomic, and distributional criteria, some authors elevated Tridentatae to subgeneric status as Tridentatae (McArthur and Sanderson, 1999). Nevertheless, this classification does not accurately represent the natural groups. Molecular phylogenetic studies, based on the analysis of chloroplast (cpDNA) and nuclear ribosomal (nrDNA) sequences have helped elucidating the systematic relationships within Artemisia, although important questions remain unresolved. Despite some differences, there is a close phylogenetic relationship between Artemisia and Absinthium, which is consistent with the available molecular phylogenies presenting species of the subgenera Artemisia and Absinthium intermixed (Pellicer et al., 2008).
Artemisia dracunculus (Subgenus: Dracunculus) investigation, A. abrotanum (Subgenus: Artemisia L. (5 Bessers’s Subgenus: Artemisia L.)), A. absinthium (Subgenus: Artemisia L. (Bessers’s Absinthium)) (McArthur and Sanderson, 1999), and A. vulgaris (Subgenus: Artemisia) (Pellicer et al., 2008) exploration revealed that volatile oils from A. abrotanum containing davanone or silphiperfolane derivatives showed the highest antifungal activity, which emphasized that Artemisia oils are promising in the development of novel anti-Candida drugs (Obistioiu et al., 2014). T. vulgaris oils are active against virulence factors and biofilms, proteinase and haemolysin producing drug-resistant strains of Candida spp. These activities are supposed to be the main contribution due to their major active compound thymol (Khan et al., 2014). S. officinalis L. essential oil exhibited antecedental activities and had inhibitory effects on the adhesion of the cells to resin surface of polymethyl methacrylate. There is a possibility to use S. officinalis essential oil as an antifungal denture cleanser to prevent candidal adhesion and to reduce the risk of Candida-associated denture stomatitis (Sookto et al., 2013). S. officinalis L. essential oil exhibited antecedental activities against C. albicans and had inhibitory effects on the adhesion of the cells to the surface of polymethyl methacrylate resin.

In Brazil, essential oils from the leaves and/or roots of 35 medicinal plants commonly used in that country were screened for anti-C. albicans activity. Essential oils from 13 plants showed antecedental activity. There was a strong activity against C. albicans of oils of Achillea millefolium, Mikania glomerata (Fam. Asteraceae), and Stachys byzantina (Fam. Lamiaeae), (MIC = 0.25 µg mL\(^{-1}\)). Aloysia triphylla (Fam. Verbenaceae), Anthemis nobilis (Fam. Asteraceae), Cymbopogon martini (Fam. Poaceae), Cyperus articulatus, Cyperus rotundus (both belong to the Fam. Cyperaceae), Lippia alba (Fam. Verbenaceae), Mentha arvensis and Mentha piperita (Fam. Lamiaeae) presented a moderate activity (MIC between 0.6 and 1.5 µg mL\(^{-1}\)). Weak activity (weak inhibitors – MIC above 1.6 µg mL\(^{-1}\)) was shown by Baccharis dracunculifolia (Fam. Asteraceae), O. vulgare, Piper regnellii, and T. vulgaris. All the remaining plants presented a MIC above 2.0 µg mL\(^{-1}\) (Duarte et al., 2005).

One Serbian study included investigation of the essential oils obtained from R. officinalis, S. officinalis, and Satureja kitaibelii (S. kitaibelii) (Fam. Lamiaeae) to test C. albicans growth in vitro. The activity of herb essential oils was expressed as MIC values against C. albicans ATCC strain 1023, MIC values for R. officinalis, S. officinalis, and S. kitaibelii were: 50 µg mL\(^{-1}\), 25 µg mL\(^{-1}\) and 12.5 µg mL\(^{-1}\), respectively. The essential oil of S. kitaibelii had the strongest inhibitory activity and the lowest MIC value of 12.5 µg mL\(^{-1}\), and probably can be the best candidate for fighting C. albicans oral infection (Tambur et al., unpublished).

In addition, testing of the antimicrobial activity of S. kitaibelii essential oil against the battery of 30 pathogenic microorganisms showed a significant activity against dermatophyte strains (Mihajilov-Krstev et al., 2011). Another investigation showed that essential oil of Teucrium montanum (Fam. Lamiaeae) had antifungal effect against three mold species: Fusarium, Aspergillus and Penicillium (Vukovic et al., 2007).
Tanideh and co-workers concluded that experimental oral *C. albicans* mucositis can be treated with *H. perforatum* extract administered orally or topically (Tanideh et al., 2014). It may be of interest to notice that *H. perforatum* endophyte *Seimatosporium* sp. (fungus) (Fam. anamorph coelomycete) exhibited a significant antifungal activity against *C. albicans* by (−)-avenaciolide as the only bioactive constituent of the extract (Clark et al., 2014).

Investigation of activity of ethanol extracts obtained from other plants such as *Acacia nilotica*, (Fam. Fabaceae), *Syzygium aromaticum*, *Cinnamon zeylanicum* (Fam. Lauraceae), *Eucalyptus globulus* (Fam. Myrtaceae), and *Terminalia arjuna* on *C. albicans* ATCC 10231 growth exhibits strong inhibitory effects of *A. nilotica* and *S. aromaticum* (Khan et al., 2014).

Several investigations revealed the antifungal activity of herbs growing in Serbia against *Candida*. Experimental oral mucositis can be treated with *H. perforatum* extract administered orally or topically (Tanideh et al., 2014). Methanol extracts of *M. piperita*, *Mentha longifolia*, *Plantago lanceolata* (Fam. Plantaginaceae), and *Artemisia austriaca* displayed some activities against *C. albicans* (Yiğit et al., 2009).

Autochthonous species have been described in a substantial number of studies. Antifungal activity of ethanol extracts of plants in the study undertaken by Rajah Muthiah Medical College and Hospital, Annamalai University, India, was confirmed in investigation on *Candida* strains (*C. albicans* was isolated in 76.08% of the oral lesions). Plants: *Syzygium jambolanum*, *Cassia siamea*, *Odina wodier* (Fam. Anacardiaceae), *Momordica charantia* (Fam. Cucurbitaceae), and *Melia azedarach* (Fam. Meliaceae) and two algal species, *Sargassum wightii* and *Caulerpa scalpelliformis* were tested against 25 strains by disc diffusion method. Antifungal activity was observed for medicinal plants *Syzygium jambolanum* and *Cassia siamea*, and for seaweed *Caulerpa scalpelliformis* at 100 µg mL⁻¹ and *Sargassum wightii* at 10 µg mL⁻¹ (Prabhakar et al., 2008).

Ethanol extracts from the leaves and/or roots of 35 medicinal plants commonly used in Brazil were also screened for anti-*C. albicans* activity. Ethanol extracts of *Achillea millefolium*, *Mikania glomerata*, and *Stachys Byzantina* (MIC = 0.25 µg mL⁻¹). *Aloysia triphylla*, *Anthemis nobilis*, *Cymbopogon martini*, *Cyperus articulatus*, *Cyperus rotundus*, *Lippia alba*, *Mentha arvensis* and *M. piperita*, *Baccharis dracunculifolia*, *O. vulgare*, *Piper regnellii* and *T. vulgaris*, and other investigated plants were not effective against *C. albicans* at any of the concentrations tested (Duarte et al., 2005).

In the study conducted in Kisii region, southwest Kenya, on the various leaf extracts of the *Carissa spinarum* (Fam. Apocynaceae), *Urtica dioica*, *Warburgia ugandensis* (Fam. Canellaceae), *Senna didymobotrya*, *Physalis peruviana*, *Bidens pilosa* (Fam. Asteraceae), *Leonotis nepetifolia* and *Toddalia asiatica*, the highest antifungal activity against *C. albicans* was noted in ethanol leaf extracts of *Leonotis nepetifolia* and *Toddalia asiatica* (Maobe et al., 2013).

Ethanol extracts of 58 tradional Chinese plants belonging to 45 families were examined for their activity against *A. Fumigatu* and *C. albicans*. The
anti-fungal activities of the selected plant extracts were tested against two kinds of fungal species, namely, C. albicans represented the yeast model and A. fumigatus represented the filamentous fungus. The activities of these extracts were evaluated in a dose-response curve with 1.00, 0.10 and 0.01 µg mL\(^{-1}\) concentrations. These results revealed that 13 plant extracts, Solanum nigrum (Fam. Solanaceae), Poria cocos (Fam. Polyporaceae), Eucommia ulmoides (Fam. Eucommiaceae), Atractylosides macrocephala (Fam. Asteraceae), Polygonum cuspidatum (Fam. Polygonaceae), Ligustrum lucidum (Fam. Oleaceae), Polygala tenuifolia (Fam. Polygalaceae), Saposhnikovia divaricata (Fam. Apiaceae), Mahonia fortunei (Fam. Berberidaceae), Cynanchum paniculatum (Fam. Apocynaceae), Lobelia chinensis (Fam. Campanulaceae), Aster tataricus (Fam. Asteraceae), and Uncaria rhynchophylla (Fam. Rubiaceae), showed a high inhibitory activity against A. fumigatus, representative of filamentous fungi. However, only two plant extracts, Codonopsis pilosula (Fam. Campanulaceae) and Tussilago farfara (Fam. Asteraceae), showed a high inhibitory effect against C. albicans.

These antifungal activities suggest that different plant extracts may contain very selective target compounds that have specific selectivity towards A. fumigatus and C. albicans. The results are very significant as most of the plants showed an inhibitory effect not only at the concentration of 1.0 µg mL\(^{-1}\) (Zhang et al., 2013).

There are plenty of herbs used in traditional medicine in South Africa. In the investigation of leaf extracts of Cussonia zuluensis (Fam. Araliaceae), Vepris reflexa (Fam. Rutaceae), Curtisia dentata (C. dentata) (Fam. Curtisiaceae), Trichilia emetica (Fam. Meliaceae), Terminalia phanerophlebia, Terminalia sambesiaca, and Kigelia africana (Fam. Bignoniaceae), extracts of C. dentata, T. sambesiaca, and T. phanerophlebia had the highest activities against fungal test organisms. The most efficient activity was observed in the acetone extracts of C. dentata against A. fumigatus, Micrococcus canis, C. albicans, Sporothrix schenckii, and Cryptococcus neoformans. The authors concluded that C. dentata is a candidate for further work on isolation of compounds active against C. albicans (Shai et al., 2008).

In a recent study, the effects of some herbs traditionally used in phytotherapy for a wide spectrum of diseases in Serbia have been evaluated (Tambur et al., in press). Ethanol extracts activity measured as MIC values for Sinapis alba (Fam. Brassicaceae), T. vulgaris, H. perforatum, Teucrium montanum, Artemisia absinthium, Plantago lanceolata, S. officinalis, C. officinalis, Acorus calamus (Fam. Acoraceae), Malva mauritanica (Fam. Malvaceae), Tilia cordata (Fam. Malvaceae), Aesculus hippocastanum (Fam. Sapindaceae), Capsella bursa-pastoris (Fam. Brassicaceae), Origanum majorana, and A. millefolium were investigated against Candida albicans ATCC strain 1023. It has been shown that extracts of Sinapis alba, Teucrium montanum, A. absinthium, Plantago lanceolata, C. officinalis, Acorus calamus, Malva mauritanica, Tilia cordata, Capsella bursa-pastoris, Origanum majorana, and Achillea A. millefolium had MIC = 300 µg mL\(^{-1}\), however, for S. officinalis and H. perforatum, MIC was > 300 µg mL\(^{-1}\) without any effect to Candida growth. The extract of T. vulgaris had MIC of 150 µg mL\(^{-1}\), while Aesculus hippocastanum had a stronger antimicrobial activity (MIC =37.5 µg mL\(^{-1}\)) (Tambur et al., unpublished).
ANTIFUNGAL ACTIVITY OF HONEY

Since honey is also of plant origin, it is not surprising that it could have the role in antifungal activity (Fallico et al., 2004; Djouahri et al., 2013). Several factors may influence the antifungal activity of honey. For example, DeMera and Angert (2004) reported that honey from different phytogeographic regions varied in their ability to inhibit the growth of yeasts, suggesting the botanical origin influencing the antifungal activity. However, there are other antibacterial factors such as high sugar or hydrogen peroxide concentration, as well as low pH, which are well known. More recently, identification of methylglyoxal and the antimicrobial peptide bee defensin-1 in honey confirmed that these substances are important antibacterial compounds, but also with strong antifungal activity against many Candida species such as C. albicans, C. parapsilosis, C. tropicalis, C. kefyr, C. glabrata, and C. dubliniensis (Khosravi et al., 2008; Moussa et al., 2012).

ANTIBACTERIAL HERB EFFECTS

Herbs actively suppress the growth of some bacteria, too. In Serbian traditional medicine, substantial number of plants that belong to several different families such as Lamiacea, Asteracea, Brassicaceae, Hypericaceae, Plantaginaceae, Acoraceae, Malvaceae, Tiliaceae, Sapindaceae, and others, have a paramount position. Scientific evidences have confirmed that some extracts of these plants act efficiently against many bacteria (Mihajilov-Krstev et al., 2011; Vukovic et al., 2007; Samoilova et al., 2014).

The essential oil of Teucrium montanum had a strong antibacterial effect against 13 bacterial species (Vukovic et al., 2007). Ruscus aculeatus L. and Ruscus hypoglossum L. showed an antimicrobial activity on pathogenic Gram-positive and Gram-negative microflora: S. aureus (ATCC 6538), B. cereus (human isolate), Micrococcus flavus (ATCC 10240), L. monocytogenes (NCTC 7973), P. aeruginosa (ATCC 27853), Enterobacter cloacae (human isolate), S. typhimurium (ATCC 13311), and E. coli (ATCC 35210). Substances isolated from these plants responsible for this effect are the same as for antifungal activities: phenolic acids such as p-coumaric and caffeic acid and flavonoid rutin pointed to the R. aculeatus herb as a potentially new promising herbal material (Hadžifejzović et al., 2013).

Investigation of autochthonous medicinal plants from the faraway Himalayas in Ladakhi region, near Siachin glacier, showed that some extracts expressed significant antibacterial effects, especially leaf samples of Podophyllum hexandrum (Fam. Berberidaceae), stem of Verbascum thapsus against Bacillus subtilis, and flower of Salvia sclarea against Pseudomonas aeruginosa (P. aeruginosa) (Kumar et al., 2010). Ethanol extracts of 58 traditional Chinese plants belonging to 45 families were examined for their activity against Acinetobacter baumannii, P. aeruginosa, and S. aureus. A total of 30 plant extracts showed significant antimicrobial activities against these test microbial strains (Zhang et al., 2013).
Some plants (*Allium sativum* L.) may ameliorate effects on gentamicin nephrotoxicity in the kidney (Nasri et al., 2013). In Pakistan, investigation of the crude extracts and fractions of six medicinal plants revealed that methanol fraction of *Pistacia integerrima* (Fam. Anacardiaceae), chloroform fractions of *Debregeasia salicifolia* (Fam. Urticaceae) and *Toona ciliata* (Fam. Meliacea), and aqueous fraction of *Aesculus indica* are suitable candidates for the development of novel antibacterial compounds (Bibi et al., 2011). In a study where twenty-one herbal extracts were screened for antiadhesive activity against *Campylobacter jejuni*, the highest anti-adhesion effects were obtained for *Zingiber officinale* (ginger) (Fam. Zingiberaceae), *Capsicum annuum* (cayenne) (Fam. Solanaceae), and *Glycyrrhiza glabra* (licorice) (Bensch et al., 2011).

Essential oil of *R. officinalis* expressed *in vitro* antibacterial effects against meticillin-resistant (MR), and meticillin-sensitive *Staphylococcus aureus* (MSSA), *Escherichia coli*, *P. aeruginosa*, *Salmonella* Typhimurium, and *Salmonella Enteritidis* (Barbosa et al., 2015). The extract from *Urtica dioica* reduced biofilm formation in *E. coli* BW 25113 (Samoilova et al., 2014). The ethanol extracts of 15 traditionally used medicinal plants against ESBL-producing drug-resistant enteric isolates demonstrated a broad-spectrum activity against all test isolates (Ahmad and Aqi, 2007).

*C. officinalis* and *Camellia sinensis* L. (Fam. Theaceae) demonstrate an antimicrobial activity inhibiting the adherence of microorganisms to sutures after extraction of unerupted third molars (Faria et al., 2011). During the evaluation of the antibacterial effects of some medicinal plants, *Thymus caramanicus* and *Zataria multiflora* (Fam. Lamiaceae) were the most effective ones against several pathogens, including *S. aureus*, *Shigella dysenteriae*, *S. typhimurium*, *E. coli*, *Staphylococcus epidermidis*, *Bacillus subtilis*, MRSA, and *P. aeruginosa* (Mahboubi et al., 2014). Investigation on *A. absintum* revealed the effects of 49,59-O-dicaffeoylquinic acid (49,59-ODCQA) as a pump inhibitor with a potential of targeting efflux systems in a wide panel of Gram-positive human pathogenic bacteria (Fiamegos et al., 2014).

Medicinal plants may be a valuable alternative to the control of bacteria in raw beef. *R. officinalis* and especially *T. vulgaris* essential oils can reduce the presence of *Listeria monocytogenes* (Oliveira et al., 2013).

It is worth mentioning that extracts of one of the species of lichen *Usnea* (lichen is a symbiosis between a fungus and an alga or cyanobacterium Hale, ME.) (Fam. Parmeliaceae), where the fungus belongs to the division Ascomycota, while the alga is a member of the division Chlorophyta), *Usnea barbata*, showed a strong antibacterial activity against the most of the tested strains of *Streptococcus*, *Enterococcus*, and *Staphylococcus* of different origin (Zizovic et al., 2012).

The acetone extracts of *C. dentata* expressed the most efficient activity. *C. dentata* extracts also had five compounds active against other tested bacterial species (*S. aureus*, *E. coli*, *Enterococcus faecalis* and *P. aeruginosa*). An opposing, microbial growth inhibitory effect of *C. dentata* extracts was non-selective (Shai et al., 2008).
CYTOTOXICITY

In addition, it may be of interest to note that evaluation of the antimicrobial activity and analysis of the cytotoxicity of Brazilian plant extracts of *Equisetum arvense* L (Fam. Equisetaceae), *Glycyrrhiza glabra* L., *Punica granatum* L. (Fam. Lythraceae), and *Stryphnodendron barbatinam* Mart. (Fam. Fabaceae) against *S. aureus*, *S. epidermidis*, *Streptococcus mutans*, *C. albicans*, *C. tropicalis*, and *C. glabrata* revealed that all plant extracts were effective against the microorganisms tested. The *G. glabra* L. extract exhibited least cytotoxicity and the *E. arvense* L. extract was the most cytotoxic (Oliveira et al., 2005). When investigation was extended to other virulence mechanisms of *C. albicans*, it was noticed that the concentration-dependent activity of *Carum copticum* (Fam. Apiaceae) and *T. vulgaris* oils exist against virulence factors and biofilms, protease and haemolysin producing drug-resistant strains of *Candida* spp. Thymol is also major active compound which play a significant role against biofilms (Khan et al., 2014).

One species of a genus of evergreen coniferous tree, *Tetraclinis articulata* (Vahl) Masters, also known as *Thuja articulata* (Vahl) or *Callitris quadrivalvis* (Vent) (Fam. Cupressaceae) (Barrero et al., 2005) is native to northwestern Africa, to Morocco, Algeria, and Tunisia. It grows also in small populations in Malta and near Cartagena (southeast Spain) (Bourkhiss et al., 2010). This tree is used in traditional and popular medicine to cure numerous infections both in childhood and adulthood: respiratory and intestinal infections, gastric pains, diabetes, hypertension and rheumatism (Bourkhiss et al., 2007; Bourkhiss et al., 2010). It was observed that this plant not only has antibacterial and antifungal effects (Bourkhiss et al., 2007; Bourkhiss et al., 2010; Djouahri et al., 2014), but it also possesses cytotoxic, antioxidative, and anti-inflammatory characteristics (Djouahri and Boudarene, 2012). Having in mind a broad spectrum of treated illnesses, the chemical composition of *Thuja articulata* (Vahl) has been successfully evaluated (Djouahri et al., 2013).

CONCLUSION

Medicinal plants are a substantial reservoir of positive effects on human health. There are records about their use in the early history, but we can assume that herbs were used for healing before the historical documents existed. Some herbs are studied in details and some of their compounds including useful ones are well-known. However, as humans, we have to be very careful with the flora generally, and with medicinal plants especially, because they are very sensitive to different influences including human behavior. In addition, we have to be very careful about our relationship with wild areas in order to preserve the natural floral ecosystems.

Medicinal plants remain a reservoir of undiscovered drugs, or substances that have antimicrobial effects. In Serbia, many healing species grow. Our
autochthonous medicinal plants are investigated to some extent, but perhaps the application of the isolated substances is not as wide as we could expect. In addition, their investigation could help the accumulation of knowledge and changing of attitude towards their use: they can be used instead of conventional drugs or together with remedies of conventional medicine. Interest in medicinal plants could influence the evolving relationship between pharmaceutical industry and pharmacognosy. Investigation of medicinal plant compounds and their effect on the target microorganisms can develop chemical synthesis or plantation of medicinal plants.

Many authors and numerous studies have shown that extracts of some plant species autochthonous in Serbia, as well as the exotic ones, can have strong antimicrobial effects. Studies coming from almost all geographic regions in the world revealed results on the application of their autochthonous medicinal plants to some pathogenic fungi such as \textit{C. albicans}.

Toothpaste ingredients can involve only chemical compounds, but some individuals prefer toothpastes with addition of herbal extracts, or only with herbal extracts in vehiculum. For that reasons, the effects of some medicinal plants to \textit{C. albicans} growth should be taken into consideration. It seems that toothpaste that contains both herbal extracts and sodium fluoride is more effective in the control of \textit{C. albicans} than toothpaste containing monofluorophosphate only. In addition, some herbal toothpastes appear to be equally effective as fluoride dental formulations. They can be an acceptable alternative in the future to the individuals who prefer natural approach in the prevention of oral cavity diseases.

ACKNOWLEDGMENTS:

This paper is supported by the Ministry of Education and Science of the Republic of Serbia (Project No 34021).

REFERENCES


ИНХИБИТОРНИ ЕФЕКАТ МЕДИЦИНСКИХ БИЉАКА НА РАСТ *Candida albicans* И БАКТЕРИЈА У УСНОЈ ДУПЉИ

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РЕЗИМЕ: У овом прегледном раду разматра се деловање етанолских екстрахата, есенцијалних уља и цитотоксичности појединих медицинских биљака које се користе у традиционалној медицини у различитим географским подручјима широм света, укључујући и Србију, на раст, умножавање и патогеност кваснице *Candida albicans*, и бактерија које имају водећу улогу у одржавању оралног екосистема. Разворсне медицинске биљке, као што су *Rosmarinus officinalis* (Fam.
Lamiaceae), *Artemisia dracunculus* и *Artemisia absinthium* (Fam. Asteraceae), на-
стањују различите географске регије, континенте као и Балканско полуострво,
међу којима су неке и аутохтоне *Hypericum perforatum* L. (Fam. Hypericaceae),
*Urtica dioica* L. (Fam. Urticaceae), *Achillea millefolium* L. (Fam. Asteraceae), *Matricaria
chamomilla* L. (Fam. Asteraceae), *Sambucus nigra* L. (Fam. Caprifoliaceae) и *Thymus
serpyllum* L. (Fam. Lamiaceae) с импресивном антимикробном активношћу у усној
дупљи.

КЉУЧНЕ РЕЧИ: медицинске биљке, *Candida albicans*, микрофлора усне
dупље, бактерије