A review of phytotherapy of *Acne vulgaris*

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Abstract

*Acne vulgaris* (acne) is a cutaneous pleomorphic disorder of the pilosebaceous unit involving abnormalities in sebum production and is characterized by both inflammatory (papules, pustules and nodules) and non-inflammatory (comedones, open and closed) lesions. *Propionibacterium acnes* and *Staphylococcus epidermidis* are common pus-forming microbes responsible for the development of various forms of acne. This disease remains a common condition in industrialized societies, with many mainstream treatment options available. There are many acne products on the market, and making an appropriate selection can be daunting.

Common therapies that are used for the treatment of acne include topical, systemic, hormonal, herbal and combination therapy. Topically used agents are benzoyl peroxide, antibiotics and retinoid. Systemically used agents are antibiotics and isotretinoin. However, all such treatments carry risks and none is completely satisfactory. Natural alternatives are gaining greater research support, and have much to offer clinically in this disorder.

This review focuses primarily on herbal treatments for acne that show scientific evidence of clinical efficacy, as well as the more common herbs shown to be useful in the treatment of this dermatologic disorder.

**Key words:** Acne vulgaris, acne treatment, herbal therapy, phytotherapy of acne

Introduction

Acne is a chronic disease of the pilosebaceous follicle that causes polymorph cutaneous lesions, among them comedones, papules, cysts, pustules, and abscesses which, after regression, may leave scars (Ramos-e-Silva and Carneiro, 2009). It is one of the most common skin diseases encountered by community physicians and dermatologists. Acne can present at any age, from neonates to mature adults, but is most prevalent and severe during adolescence, reaching a peak at the age of 14-17 years in females and 16-19 years in males (Lucky, 1998; Williams and Layton, 2006; Rivera, 2008).

The distribution of acne corresponds to the highest density of pilosebaceous units (face, neck, upper chest, shoulders, and back). Acne classification, scarring, acne rosacea, chloracne, acne associated with polycystic ovary syndrome, infantile acne and acne inversa have been reviewed elsewhere (Jacob et al., 2001; Shalita, 2004; Meixner et al., 2008).

Depending on the appearance different types of acne can be distinguished: *a)*. blackheads, which are open comedones, where the top of accumulated sebum in the follicle opening oxidise and appears gray or black; *b)*. whiteheads, which are closed comedones, where the follicle opening is clogged with trapped sebum and sealed by normal colored skin; *c)*. cystic acne, tender, hard, purple lumps often larger, and fluid-filled inflammatory swellings deep in the skin; *d)*. acne scars, after healing of cystic acne small, depressed pits (acne scars) and pigmentation is left behind; *e)*. acne rosacea, features redness (caused by dilation of small blood vessels); *f)*. acne pimples, mainly on the cheeks and forehead, common in women in middle life.
and g). acne vulgaris, different kind of pimples and blemishes (papules, pustules, nodules) which are pus-filled and inflamed firm spots below the skin (Shalita, 2004).

However, the term acne in medical circle is correlated to common acne (Acne vulgaris). In addition to adolescent acne, drugs are a relatively common cause of eruptions resembling acne. The most common are steroids, androgenic hormones, certain anti-convulsives, anti-tuberculosis drugs, lithium, and others. Exposure to iodides, bromides, and chlorides has also been reported to cause acne (Valeyrie-Allanore et al., 2007). Drug-induced acne or acneiform dermatoses that can have a sudden onset e.g. within one day of drug administration can be resolved after the drug is stopped. Acneiform dermatoses have an unusual lesion distribution, such as inflammatory papules and pustules that are small and uniform in size (monomorphic), and can lead to secondary comedones of which the earliest histological event is spongiosis followed by lymphocytic and neutrophilic infiltrates, respectively (Plewig and Jansen, 1998; Momin et al., 2009). Therefore, although the initial causes are different, the pathogenesis of Acne vulgaris can be similar.

The pathophysiology of acne is slowly unraveling, and although many factors remain undetermined, a better understanding of the mechanisms involved has led to an improvement in acne management over the last two decades. Four key factors have been identified in the etiology of acne: increased sebum production, follicular hyperkeratinization, colonization of the pilosebaceous unit with Propionibacterium acnes (P. acnes) and the production of inflammation (Kurokawa et al., 2009). Sebum hypersecretion in deformed follicles leads to formation of microcomedones, and the follicular hyperproliferation of microcomedones causes inflammation, and comedones in both open and closed types (black and white comedones) appearing in papules, pustules, nodules and cysts. The resulting skin condition with sebum enrichment is prone to the anaerobic growth of P. acnes, which is the main causative microorganism in acne. In addition, Staphylococcus epidermidis and Pitrosporum ovale are present in acne lesions. Proliferation of these microorganisms, mainly P. acnes, leads to inflammatory lesions and severe acne (Leyden, 2001; Zaenglein and Thiboutot, 2006; Morelli, 2007).

Common acne treatments

Acne needs to be managed aggressively from the outset using a combination of treatments directed against each of the relevant factors.

Generally, the choice of acne therapy is largely determined by the severity and extent of the disease. According to the type and severity, acne is often graded on a scale from mild-to-moderate inflammation, featuring predominantly comedones, erythematous papules to papulo-pustules, to moderate-to-severe papulo-nodular, nodulo-cystic and scarring inflammatory states (Olutunmbi et al., 2008). However, defining optimum treatment strategies remains difficult as significant variability exists between individuals, both in terms of clinical presentation (disease duration, predisposition to scarring and post inflammatory hyperpigmentation) and response to previous treatments.

In approximately 60% of cases, acne is a self-limiting condition that can be managed with combination treatment followed by topical maintenance therapy (Thiboutot et al., 2009). In other cases, acne follows a chronic course that requires treatment for a prolonged period. Even mild acne can persist for 4-6 years, and in severe cases, the natural history could be in excess of 12 years (Gollnick et al., 2008). The reason as to why acne becomes chronic in some patients is not well understood, and predicting which patients will have persistent and/or refractory acne is very difficult. Factors that link to poor prognosis include early onset, hyperseborrhea, truncal acne and scarring (Dreno et al., 2006). A logical understanding of the pathophysiology of acne and the impact of therapies on these etiological factors should form the foundation of any treatment selection.

For mild and moderate acne, over-the-counter (OTC) and prescription medications may be the only treatment required. The most frequently used topic substances in acne treatment are retinoids, benzoyl peroxide, antibiotics, anti-seborrheic medications, salicylic acid, alpha hydroxy acid, azelaic acid, nicotinamide, and keratolytic soaps (Gollnick and Krautheim, 2003; Ramos-e-Silva and Carneiro, 2009). Oral medications are used in severe cases, when an inflammatory component is present and in topical resistant cases. The most frequently prescribed are antibiotics, isotretinoin, and hormones. In very severe inflammatory cases it may be necessary to use systemic corticoids. Systemic treatment can be used even in mild cases, if there is intolerance to the topical treatment or where topical therapy has failed. Basic topical and systemic protocols may include several therapies, used according to the severity of each case (Auffret, 2000; Usatine and Quan, 2000; Bershad, 2001; Oberemok and Shalita, 2002).

However, these drugs produce a number of potential side effects and development of resistance to frequently used antibiotics. This leads to treatment failure with previously used successful therapy. Therefore, an alternative for the treatment of acne have been studied and developed and as a result natural approaches to combating acne and its disfiguring effects have gained popularity. Numbers of conventional and novel herbal cosmetics are useful to treat damaged skin (Amit et al., 2007; Ashawat et al., 2007; Chanchal and Swarnlata, 2008).

Acne can be cured by herbs either consuming internally or externally or with both. Topical herbal treatment is preferable choice of consumers as ease of application and it surpasses the bitter taste of herbal formulation (when taken internally). Because herbs are safe, efficacious and the added advantage of multi functionality, herbs are increasingly being used in mainstream cosmetic	

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products, including acne-fighting compositions (Aburjai and Natsheh, 2003; Chanchal and Swarnlata, 2008; Kumar et al., 2008). Although some of the herbs are scientifically explored for their efficacy in treatment of acne but still many herbs are remain untouched by scientist.

This review focuses on the benefits of some herbs for the treatment of Acne vulgaris. The purpose of this study is to open new avenues and set trends for the improvement of medicinal uses of herbs for acne treatment and also reflects the traditional knowledge which provides the base for clinical research to be carried out to explore the active compounds which are responsible for anti-acne activities.

**Herbal therapy of acne**

The quest for medications and cosmetic measures to combat acne continues to be a major research and development initiative in the pharmaceutical and cosmetic industries. Number of herbs with a history of use in traditional cultures has entered in the growing cosmetic market.

Herbal formulations which contain many herbal extracts and have negligible adverse effects compared with modern medicines are commonly indicated for moderate and severe forms of acne. The efficacy of these agents in acne treatment is not only based on antimicrobial activity but also on their possessed antioxidant and anti-inflammatory properties by which they inhibit neutrophile migration and generation of reactive oxygen species. Also, various herbs are used in acne due to their skin detoxification property. Herbal extracts or oils may be used as monotherapy or in combination therapy. Till now, there are certain herbal extracts such as *Angelica dahurica*, *Melaleuca alternifolia*, *Azadirachta indica*, *Rhizoma coptidis* and *Psidium guajava*, that are proved to be more effective that anti-biotics and retinoids in acne therapy (Kumar et al., 2008). Below some herbs are discussed in details for their potential efficacy in acne treatment.

*Arctium lappa* (Great burdock)

Burdock root extract has strong antibacterial and estrogenic effect and therefore it is used for treatment of different skin conditions like eczema, psoriasis, and acne.

The root of the burdock plant has been used in its native haunts, which include much of Africa and Europe, to improve immunity and overall health for at least 3,000 years. Like the honeybee, it has followed civilization. The plant produces a burr that gets stuck on people’s clothing, and in this manner it has been carried to every continent. In recent years, the burdock has come to be considered a weed, despised by lawn owners for its tenacious growth habits.

The plant is a biennial, meaning that in its second year it blooms and then dies. Burdock spends its first year of life working industriously to store all the necessary elements to bloom the following year. The root is considered to be the most important part of the plant. It is usually plucked from the ground in the autumn of the plant’s first year just as leaves started to fall.

The root contains lignans including arctigenin, glyco-

![Fig. 1. Main constituents of *Arctium lappa* (Great burdock) (http://www.medicinescomplete.com/mc/herbals/current/images/HrbburdockC001_default.png).](image-url)
side arctiin, and mataresinol, polyacetylenes including tri-decadienatetraynes, tridecatrienatryenes, and a sulfur containing arctic acid (Fig. 1) (Park et al., 2007). It also contains amino acids including alpha guanidino-n-butyric acid, inulin, organic acids, fatty acids, and phenolic acids (Wang and Yang, 1993; (Community herbal monograph on A. lappa, radix, EMA/HMPC/246763/2009 Corr.1).

In general, burdock has the ability to gently stimulate health and, as a consequence, to improve the appearance of the skin. Elements contained in the plant improve the digestion and absorption of food, which makes the body stronger and better able to fight with infections. Furthermore, one of main attributes of burdock is its detoxification ability. The elimination of toxic substances via the urine is also aided by the burdock due to its mild diuretic property. Beyond its general health-stimulating abilities and like many other members of the daisy family, chamomile, elecampane, and calendula included, burdock is also considered to be one of the best tonic correctives of skin disorders. Burdock is a classic remedy for skin conditions which result with dry, scaly skin and cutaneous eruptions, eczema, psoriasis, dermatitis, boils, carbuncles, styes and chronic acne. Whereas calendula is only used externally to improve the skin’s appearance, burdock has been recommended for internal (burdock tea, tincture, fluid extract, capsules) and external use (ointment, mask) in skin disorders.

Biological activities and pharmacological functions reported for burdock include anti-inflammatory, anticancer, anti-diabetic, diuretic, antimicrobial, antiviral and free radical scavenging activities (EMA, HMPC, 2009). The Committee for herbal medicinal products from European Medicines Agency (EMA) besides use as an adjuvant in minor urinary tract complaints and for improvement of appetite, recommended root from this plant for treatment of seborrheic skin conditions (Community herbal monograph on A. lappa, radix, EMA/HMPC/246763/2009 Corr.1).

Its antibacterial (Pereira et al., 2005; Gentil et al., 2006), anti-inflammatory (Lin et al., 1996; Zhao et al., 2009) and antioxidant properties are particularly beneficial in acne treatment. Studies have shown that burdock root is able to inhibit the growth of the acne causing P. acnes bacteria which is found naturally in sebum. Also, burdock is rich with essential fatty acids, which contribute in regenerative processes in the skin. Burdock root is also able to regulate the function of the sebaceous glands, which are responsible for sebum production; the natural oil which builds inside clogged pores to create acne blemishes. On the other hand, in traditional Chinese medicine acne or eczema are seen as symptoms of system intoxication. Therefore, burdock and its clinically proven ability to act as a diuretic could effectively resolve problems affecting the skin.

**Oenothera biennis** (Evening primrose)

Evening primrose oil (EPO) made from the seeds of *O. biennis* is a fixed oil extremely rich in essential fatty acids playing an important role in prostaglandin synthesis of human body. Prostaglandins help to regulate the action of several hormones like estrogens and have anti-inflammatory action. EPO has been used for a wide range of skin conditions such as eczema, psoriasis, and acne. It is also used as a dietary source of essential fatty acids and in the production of soaps and cosmetic ingredients. EPO has demonstrated significant effect in treatment of other diseases like asthma, rheumatoid arthritis, breast problems and metabolic disorders (Bayle and Usatine, 2009; Coffey, 1993; Hederos and Berg, 1996; Horrobin, 2000; Williams, 2003; Worm and Henz, 2000).

*O. biennis* is a member of evening primrose family (Onagraceae). It is found in fields, roadsides, prairies and waste places in the United States and south Canada, but it is widely naturalized elsewhere in temperate and subtropical regions. *O. biennis* is a biennial with large yellow flowers. In its first year, it forms a rosette of basal leaves. A tall flowering stem is formed in the second year. Plants produce one or two new flowers every evening.

Although the entire plant is edible, the flowers are added in salads, leaves eaten like greens, and the roots boiled like potatoes, it is primarily a minor oilseed crop used to produce the EPO. Seeds from *O. biennis* contain 14% of EPO which usually contains 50 - 70% *cis*-linoleic acid (LA) and 7-10% *cis*-gamma-linolenic acid (GLA) (Fig. 2). Wild varieties of *O. biennis* contain highly variable amounts of LA and GLA. However, extensive cross-breeding has produced a commercial variety that consistently yields oil with 72% LA and 9% GLA. Also found are *cis*-6,9,12-octadecatrienoic acid, small amounts of oleic, palmitic, and stearic acids and steroids (campesterol, and beta-sitosterol). Mucilage and tannin in the plant parts have been also analyzed (http://www.drugs.com/npp/evening-primrose-oil.html).

GLA is essential for healthy skin functioning and is produced in human body from LA. Metabolites formed from GLA improve cellular membrane function and restore the skin lipid barrier, leaving it more hydrated, moisturized and protected from injury or stress. Because of its ability to dilute sebum production, EPO is effective at calming the skin lipid barrier, leaving it more hydrated, moisturized and better able to fight with infections. Furthermore, one of main attributes of burdock is its detoxification ability. The elimination of toxic substances via the urine is also aided by the burdock due to its mild diuretic property. Beyond its general health-stimulating abilities and like many other members of the daisy family, chamomile, elecampane, and calendula included, burdock is also considered to be one of the best tonic correctives of skin disorders. Burdock is a classic remedy for skin conditions which result with dry, scaly skin and cutaneous eruptions, eczema, psoriasis, dermatitis, boils, carbuncles, styes and chronic acne. Whereas calendula is only used externally to improve the skin’s appearance, burdock has been recommended for internal (burdock tea, tincture, fluid extract, capsules) and external use (ointment, mask) in skin disorders.

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1988). Other workers have reported that EPO oral supplementation could significantly improve skin problems in patients undergoing hemodialysis mainly due to the assumption that abnormalities in plasma composition of essential fatty acids may be associated with the etiology of uremic skin symptoms, like dryness, pruritus and erythema. After six weeks of therapy with EPO, significant increase in plasma dihomo-gamma-linolenic acid, a precursor of anti-inflammatory prostaglandin E1 was observed, suggesting that oral supplementation with EPO could restore deranged plasma essential fatty acids and ameliorate skin symptoms (Yoshimoto-Furuie et al., 1999). Other workers have reported that the EPO therapeutic effect in atopic dermatitis patients with dry scaly skin lesions was associated with the normalization of serum gamma-interferon levels (Yoon et al., 2002).

Topical treatment with EPO was also beneficial in treatment of atopic dermatitis. A meta-analysis of randomized, placebo-controlled clinical trials of Efamol® (pure evening primrose oil) in atopic eczema have shown that the oil has a simultaneous, beneficial effect on itch/pruritus, crusting, edema and redness (erythema) that becomes apparent between 4 and 8 weeks after treatment was initiated (Morse and Clough, 2006). It is also important to notice that the use of EPO for management of atopic dermatitis is considered as safe and effective (Sanapati et al., 2008).

EPO has been also studied for its ability to calm and reduce inflammation due to the fact that in the human body GLA is converted to powerful prostaglandins that have potent anti-inflammatory and anti-irritant activities, protecting the skin from the damaging effects of UV radiation that lead to inflammatory skin conditions as well as skin aging. Studies on immunomodulatory and antiinflammatory activities showed that EPO proved useful effects in old age when delta-6-desaturation (delta-6-desaturate acts in the metabolism of linoleic and alpha-linolenic acid) activity decreases (Biagi et al., 1988; Charnock, 2000). Animal studies have shown that EPO stimulates COX-1 expression in some tissues (Fang et al., 1997), reduced platelet hyperaggregability in rabbits fed an atherogenic diet (De La Cruz et al., 1997) and GLA modulate the level of serum interferon-gamma, monocyte chemotactic protein-1 and tumor necrosis factor-alfa which may be a worthwhile line of treatment in certain human diseases (Dirks et al., 1998; Ismail et al., 2008). Also, experiments were performed to see the effect of O. biennis oil on antioxidant potential, given with hyperlipemic diet to New Zealand rabbits. It was observed that glutathione peroxidase activity reduced and the activities of glutathione reductase and transferase increased (De La Cruz et al., 1999). Also, EPO has shown antimicrobial activity against Staphylococcus aureus (Borchardt et al., 2009).

**Viola tricolor (Heartsease)**

Heartsease is a small plant of creeping and ramping habit, reaching at most 15 cm in height, with flowers about 1.5 cm in diameter. It grows in short grassland on farms and wasteland, chiefly on acid or neutral soils. It is usually found in partial shade. It flowers from April to September. The flowers can be purple, blue, yellow or white. They are hermaphrodite and self-fertile, pollinated by bees.

**Viola tricolor herba cum flore** contain different classes of secondary metabolites such as:

- Flavonoids. The quantity of flavonoids in the herb Viola tricolor and Viola arvensis was found to be 2.1% and 1.3%, respectively. The main flavonoids of Viola tricolor are violanthin and rutin (quercetin 3-rutinoside) (Fig. 3), together with quercetin, luteolin and luteolin 7-glucoside. Other flavonoids: apigenin mono-C-glucosides: vitexin and isovitexin (saponaretin), luteolin mono-C-glucosides: orientin and isoorientin, and scoparin (3’-O-methyl-luteolin 8-Cglucoside), and few other O- or C-glycosides (Assessment report on Viola, EMA/HMPC/131735/2009).

- Sixteen flavonoid glycosides have been separated from the methanol extract of wild pansy by microliquid chromatography: four flavonol O-glycosides of kaempferol, quercetin, and isorhamnetin; nine flavone C-glycosides of luteolin, chrysoeriol and apigenin, and three flavone C, O-glycosides of apigenin (Toiu et al., 2007; Vukics et al. 2008a; Vukics et al. 2008b; Vukics, 2009).
- Polysaccharides. The mucilage content in wild pansy herb is about 10%. Hydrolysis of polysaccharides results in glucose (35.1%), galactose (33.3%), arabinose (18.1%), rhamnose (8.4%), uronic acid (6.2%) and xylose (5.1%) residues (Assessment report on *Viola*, EMA/HMPC/131735/2009). The water soluble fraction of polysaccharides is composed of glucose, galactose and arabinose residues (2:1.8:1.1) and galacturonic acid, rhamnose and xylose. The pectin fraction contains galacturonic acid, glucose, and galactose (Assessment report on *Viola*, EMA/HMPC/131735/2009). According to Deters, the polysaccharides of wild pansy are mainly composed of galactose, glucose, galacturonic acid (34:29:27), whereas arabinose, rhamnose and mannose are minor components (7:2:1) (Deters et al., 2005).
- Phenolic acids. The content is about 0.18%, including trans-cafeic, p-coumaric, gentisic, protocatechuic, phoxybenzoic, p-hydroxyphenylacetic, and vanillic acids, and 0.06% to about 0.3% salicylic acid and its derivatives, such as methyl salicylate and violutoside (violutin, glucosidoarabinoside of methyl salicylate), and monotropitoside (primveroside of methyl salicylate) (Assessment report on *Viola*, EMA/HMPC/131735/2009).
- Volatile oil. The content is reported with 0.0086%, containing methyl salicylate as a principal constituent (Assessment report on *Viola*, EMA/HMPC/131735/2009).
- Carotenoids. In wild pansy flowers occurs cis-vi-o-laxanthin (Szabolcs and Toth, 1970). Yellow blossoms yield carotenoids (9.69 mg/g dry weight), mainly 9-cis-violaxanthin (51.3%), all-trans-violaxanthin (29.6%), 13-cis-violaxanthin (1.7%), 15-cis-violaxanthin (0.6%), antherexanthin.
- Anthocyanins. Main pigment which is responsible for the violet colour of flowers of *Viola tricolor* is composed essentially of violanin (ca 33%), a derivative of delphinidin with D-glucose, L-rhamnose, p-coumaric acid, and 2.7 to 4% of potassium (Assessment report on *Viola*, EMA/HMPC/131735/2009).
- Cyclotides (macrocyclic peptides) and other constituents (Assessment report on *Viola*, EMA/HMPC/131735/2009).

The traditional use of heartsease goes back to ancient times. Heartsease preparations were used during the Middle Ages mainly as a remedy for various skin ailments and were mentioned according to Madaus (1938) by Lonicerus 1564; Hieronimus Bock 1565, Matthiolus (1501-1577) and Andreas Caesalpinus (died 1602). Its therapeutic activity is presented in Madaus “Lehrbuch der Biologischen Heilmittel” (1938) and Jaretzky’s “Pharmakognosie” (1937). The traditional use of heartsease in different diseases has been thoroughly documented in several handbooks and in folk tradition (Allen and Hatfield, 2004; Assessment report on *Viola*, EMA/HMPC/131735/2009). Laboratory experiments have confirmed that *Viola* extract exerts antimicrobial activity against gram positive and gram negative bacteria as have anti-inflammatory and other beneficial effects.

**Antioxidant activity.** Mantle et al. (2000) compared relative antioxidant activities of different British medicinal plants, *Viola tricolor* L. included. Antioxidative activity of the plants was tested through competitive scavenging of the ABTS (2,2’-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid)), presented in terms of mM Trolox equivalent – mM TE) or O2 radicals (estimated as superoxide dismutase – SOD activity) in vitro. Antioxidant activity (mM TE/g dry weight) of fresh tissue *Viola tricolor* leaf was 1.46±0.32, whereas for flowers was 1.43±0.26. This activity was quite potent, as comparable extracts of *Ginkgo biloba* gave values of 0.62 and 0.61 mM TE/g dry weight, respectively (Vukics et al., 2008b). Therefore, authors concluded that heartsease, especially its flower, is a promising source of natural antioxidants. In addition, a significant correlation was found between the flavonoid content and antioxidant activity.

![Fig. 3. Main flavonoids in *Viola tricolor herba cum flore* a) Violanthin, b) Rutin.](image-url)
**Antibacterial activity.** The infusion, decoction and ethanol extract of *Viola tricolor* herb displayed significant inhibitory activity against *Staphylococcus aureus*, *Bacillus cereus*, *Staphylococcus epidermidis* and *Candida albicans* and moderate activity against *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Escherichia coli* and *Klebsiella pneumoniae*. The dichloromethane, ethyl acetate and methanolic fractions obtained by partitioning of Soxhlet of dried plant material, showed the lower activity. The higher activity of the extracts containing complexes of components of the plant, relative to that of the fractions comprising compounds of different polarity, suggested a synergism in antibacterial action between compounds of heartsease (Witkowska-Banaszczyk et al., 2005).

**Anti-inflammatory activity.** The anti-inflammatory activity of the tincture from *Viola tricolor* aerial parts was tested in acute inflammation induced with oil of turpentine (i.m. 0.6 ml/100 g b.w.) in male Wistar rats. The results were compared with those from a positive control group with experimental inflammation and with those of a group treated with diclofenac (30 mg/100 g b.w.). *Viola tricolor* extract (50 mg tincture/100 g b.w.) significantly reduced polymorphonuclear leukocytes and monocytes percentages and the activation of circulating phagocytes (Toiu et al., 2007).

Experimental preclinical data confirmed antioxidant, antibacterial and anti-inflammatory activity of heartsease in different skin conditions. Results from *in vitro* antimicrobial activity of *Viola tricolor* extracts support the traditional use of heartsease even though the effects are relatively weak compared to standard antibiotics.

None clinical studies were published on mono-preparations of heartsease. Randomized, double-blind, vehicle controlled study of an ointment composed of *Mahonia aquifolium*, *Viola tricolor* and *Centella asiatica* was performed on 88 patients between 18-65 years of age with mild to moderate atopic dermatitis. They were treated for 4 weeks with an ointment containing *Mahonia aquifolium*, *Viola tricolor* and *Centella asiatica* alcohol extracts (5g of each /100 g of ointment). After 4 weeks of topical treatment the primary (erythema, oedema/population, oozing/crust, excoriation and lichenification) and secondary (pruritus, global assessment of effectiveness and tolerability) endpoints were evaluated. No significant differences were observed between ointment containing *Mahonia aquifolium*, *Viola tricolor* and *Centella asiatica* alcohol extracts and the base. However, a sub-analysis indicated that the formulation might be useful under conditions of cold and dry weather (Klövekorn et al., 2007).

According to European community herbal monograph, indication for traditional use of *herba cum flore of Viola tricolor*; *V. arvensis* and *V. vulgaris* is for symptomatic treatment of mild seborrhoeic skin conditions (Community herbal monograph on *Viola tricolor*, *herba cum flore*, EMA/HPMC/131734/2009).

**Vitex agnus castus (Chaste tree)**

*Vitex agnus castus* (Verbenaceae) commonly known as chaste tree, chaste berry, or monk’s pepper is a native of the Mediterranean region. It is a small deciduous tree that grows in Asia, Europe and North America. It bears slender spikes of violet blue, 8-10 cm flowers. Locally, the plant is used as insect repellent and insecticide. A wide range of medicinal applications are also shown by other plants of this family as berries are considered as tonic supplement for male and female reproductive system.

No single constituent has been identified as being the active one, in fact, with the exception of agnoside, all constituents are found in other plants. The total sum of constituents appears to generate a synergistic effect.

- **Flavonoids:** castican, orientin, isovitexin, vitexin.
- **Iridoid glycosides:** agnoside (the reference constituent for standardization), aucubin.
- **Volatile oil (0.8-1.6%):** terpenoids (cineole, sabine, limonene, camphene), α- and β-pinene.
- **3-Ketosteroids:** *Vitex* has been found to contain 3-ketosteroids (probably progesterone and 17α-hydroxyprogesterone) by thin-layer chromatography (Russo and Galletti, 1996).

The flowers and leaves may also possibly contain progesterone, 17-hydroxyprogesterone, testosterone, and estrogens although further research is needed. Other constituents in the flowering tops include flavonoids (particularly C-glycosides), and iridoids (aucubin, agnoside, eusostoside), 3-ketosteroids, essential oils (0.8-1.6%): o-cymol, β-famescene, α- and β-pinene, cineol, sabine, limonene (Fig. 4) (Russo and Galletti, 1996).

Very often, acne flare-ups are related to the impending onset of menstruation. This particular type of acne highlights the fact that acne is often affected by hormone balance in the body. Much work has focused on the potential negative impact of androgenic hormones on acne; estrogen and progesterone can definitely also be involved. *Vitex* and *Serenoa repens* (saw palmetto) are most commonly used herbs for addressing hormonal issues that arise in acne. Studies have shown that the whole fruit extract of *Vitex* increases progesterone levels and decreases estrogen levels by acting upon follicle-stimulating hormone and luteinizing hormone levels in the pituitary gland, and decreases exceedingly high menstrual prolatin levels via dopaminergic mechanisms (Bone, 1994). This may explain the benefit of *Vitex* in improving hormonal acne conditions.

In one placebo controlled trial of males and females, after 3 months of treatment with *Vitex*, both groups experienced a 70% improvement in their acne. This was significantly better than the placebo. However, it should be noted that if *Vitex* is given to patient who does not have a relative progesterone deficiency, acne condition could be worse, and in fact may be initiated by *Vitex* use (Gardiner, 2000).
Preliminary German research also confirmed that chastetree can be effective in moderate hormonal acne. For optimal anti-acne effects, chastetree should be taken throughout the menstrual cycle. Vitex is often used together with vitamin B6, which has also proven to be quite helpful for resolving hormonal acne, although one comparative trial found that Vitex was superior to vitamin B6 for helping patients with symptoms of premenstrual syndrome (Yarnell and Abascal, 2006).

As it was mentioned previously, conditions such as acne and seborrhoea (excessive secretion of sebum) result from the action of androgens on the skin. The severities of these effects are dependent upon androgen production by the ovary or adrenal gland and the bioavailability of androgen to peripheral tissues. This in turn is related to transport of plasma androgens by specific binding proteins and to peripheral metabolism of testosterone and androstenedione to the more potent dihydrotestosterone (DHT) (Reed and Frans, 1988). An effective anti-androgen is one which blocks the androgen receptor-mediated actions of testosterone and DHT on skin. Although no actual clinical data are available, Saw Palmetto (Serenoa repens) extract is believed to be beneficial for topical use in anti-acne formulations. In vitro studies have shown that saw palmetto extract could inhibit both isoforms of the 5-alpha-reductase (enzyme that catalyze the conversion of testosterone to DHT), as well as binding of testosterone or DHT to androgen receptor (Bayne et al., 2000).

Other well-documented anti-androgenic herb is Glycyrrhiza glabra (licorice), although it also has not been studied for acne in clinical trials. Other hormone-balancing herbs may have a role in Acne vulgaris, including but not limited to, Medicago sativa (alfalfa), Chamaelirium luteum (false unicorn root), Verbena spp. (vervain), and Mitchella repens (partridge berry) (Yarnell and Abascal, 2006).

Among hormone-like effects in treatment of acne, the antibacterial activity of extracts of Vitex was tested against clinical isolates and drug resistant bacterial strains. The minimum inhibitory concentrations (MICs) of the extracts ranged between 0.312 and 5 mg/ml. Among all the extracts, the ethyl acetate was found to be most active against all the tested bacterial species (Methicillin resistant Staphylococcus aureus (0.312 mg/ml), carbapenem resistant Acetobacter baumannii (0.625 mg/ml), ciprofloxacin resistant E.coli (0.625 mg/ml), Proteus vulgaris (2.5 mg/ml), Salmonella typhi (5 mg/ml), Escherichia coli (2.5 mg/ml), Enterococcus durans (0.625 mg/ml) and Pseudomonas aeruginosa (2.5 mg/ml)). Compare to standard streptomycin ethyl acetate extract showed good activity against all the three tested drug resistant bacteria. The present study indicates that the plant contains potential anti-bacterial components such as flavonoids, terpenoids and steroids that may be of use for development of phytomedicine for the therapy of tested bacterial diseases. The results of this study demonstrated that, ethyl acetate extract from the leaves of Vitex agnus-castus showed dominant antibacterial activity against potent clinical pathogens (Arokiaraj et al., 2009). Research studies also confirmed the antifungal activity of seeds of Vitex negundo (Sathiamoorthy et al., 2007; Shaukat et al., 2009) and antioxidant and anti-inflammatory activities of methanol extract of the plant standardized on the content of flavonoids (Kulkarni et al., 2008), which effects could be also beneficial in treatment of various skin diseases.

Hamamelis virginiana (Witch hazel)

Hamamelis virginiana L. is a winter-flowering shrub, commonly known as witch hazel that is native from Nova Scotia, Canada to Texas and Florida in the U.S. It is best known for its decorative and fragrant yellow flowers and its bright yellow fall foliage. Native Americans first learned how to use witch hazel for medicinal purposes when they used the extract to relieve bleeding, swelling, bruising and discomfort of external wounds. Hamamelis virginiana was also used in sweat remedies for skin problems.
lodge to soothe sore muscles. Native Americans also considered it as an astringent and purifier and a remedy for treating tumors. Throughout American history, uses for witch hazel have included treating insect bites and stings, rashes, hemorrhoids, sores, diarrhea and dysentery. Today, the external use of witch hazel is well known for the astringency associated with the tannin content of its leaves and bark.

The main characteristic constituent of *Hamamelis virginiana* is hamamelitannin (Fig. 5), a mixture of the α- and β-forms of (2, 5-di-O-galloyl-hamamelose), its molecular structure bears two gallate moieties and a sugar unit, hamamelose (Tourino et al., 2008). Wang et al. (2003) developed an HPLC method for the determination of hamamelitannin, catechins, and gallic acid from witch hazel bark, twig and leaf. The concentrations in the bark for hamamelitannin, gallic acid, (+)-gallocatechin, and (+)-catechin were 4.77, 0.59, 0.22, and 0.39% (w/w), respectively. Hamamelitannin and catechins were also detected in the leaves at concentrations of < 0.04% (w/w).

![Hamamelitannin](image)

**Fig. 5.** Main tannin of *Hamamelis virginiana* bark.

According to Vennat et al. (1992), proanthocyanidins, phenolic acids and flavonoids have been identified in leaf extracts. Hydroxycinnamic acids and flavonoids (e.g. myricetin, leucodelphinidin, quercetin, kaempferol, and gallic acid) are found mainly in the leaves of *Hamamelis virginiana*. Phenolic compounds from leaves of *Hamamelis virginiana* were studied by Sagareishvili et al. (1999), where kaempferol, quercetin, trifolin, kaempferol 3-O-β-D-glucuronide, quercetin 3-O-β-D-glucuronide were isolated.

According to Engel et al. (1998), the composition of the volatile fraction obtained by water distillation from the leaves and bark of *Hamamelis virginiana*, and determined in detail by GC-MS, consists in about 175 (leaves) and 168 (bark) identified compounds or at least partly characterized on the basis of a computerized database (SeKoMS). The dominating substances were represented by a homologous series of alkanes, alkenes, aliphatic alcohols, related aldehydes, ketones, and fatty acid esters. Significant differences in the terpenoid and phenylpropanoid patterns of the products obtained from the bark and leaves are apparent: whereas the product of bark distillation was found to typically contain phenylpropanoids and mainly sesquiterpenoids, that obtained from the leaves included some distinct monoterpenoids detected in comparably higher amounts.

The chemical composition of the volatiles, when taken together with the absence of specific accumulation sites of lipophilics, emphasizes the definition “volatile fraction” rather than essential oil (Assessment report on *Hamamelis virginiana*, 2009).

Extracts from witch hazel bark have long been used in therapy of skin diseases and in cosmetic formulas (skin lotions, nourishing creams, pre- and after-shaves, etc.). When applied topically, witch hazel could significantly reduce bacteria growth, thus preventing inflammation and acne formation. Also, the tannin content in witch hazel has strong astringent as well as antioxidant properties. These astringent properties are cleansing to the skin, while minimizing the size of skin pores. Unlike many harsh commercial acne formulations, it is gentle, non-irritant and non-drying when used to tone and cleanse acne-infected or acne-prone skin. Furthermore, it helps to prevent any further infection from occurring. The tannins in witch hazel tighten pores and swollen veins, as well as reduce inflammation. The anti-inflammatory properties are further increased by the flavonoids and procyanidins, as well as resin, in the witch hazel plant.

Many acne treatments can irritate the skin, causing soreness, inflammation and dryness. Witch hazel, being a natural product, is well suited for skin care, because it does not disrupt the pH of the skin, which tends to cause irritation (Assessment report on *Hamamelis virginiana*, 2009).

Hamamelis extracts and isolated chemical constituents have shown anti-inflammatory activity in vitro and in vivo. It was found that polyphenols isolated from hamamelis stem and twig bark inhibited the synthesis of platelet-activating factor in human polymorphonucleocytes (PMNs). Dimeric galloylated proanthocyanidins showed the strongest effects. The synthesis of leukotriene B4 in PMNs was inhibited by the tested substances. Oligomeric proanthocyanidins had stronger activity than hamamelitannin (Hartisch et al., 1997). According to Deters et al. (2001), polysaccharides and proanthocyanidins from hamamelis bark could influence on human skin keratinocyte proliferation and differentiation of cultured human keratinocytes, and influence on irritated skin. While the polysaccharide fraction, consisting mainly of arabins and arabinogalactans, did not have effect human keratinocytes, the proanthocyanidins strongly increased the proliferation of the cells, while the differentiation was not influenced significantly. Within a preliminary cumulative in vivo study on SLS-irritated skin, proanthocyanidins were proven to reduce transdermal water loss and erythema formation. Furthermore, a clinical scoring indicated that procyanidins can influence irritation processes significantly.

An aqueous ethanolic extract of hamamelis bark (ethanol 70%) showed a significant anti-inflammatory effect (43% inhibition of oedema; p<0.05) in the croton oil ear oedema test in mice when applied topically at 250μg per ear. This effect was shown to be mainly due to proanthocyanidins of molecular weight ≥ 3kDa (69% inhibition at
250μg per ear; p<0.05) obtained from this extract subjected to ultrafiltration and identified by TLC, HPLC. Proanthocyanidins also exhibit significant antiviral activity against Herpes simplex virus type 1. In addition, the UV-concentrate displayed radical scavenging properties, inhibited α-glucosidase as well as human leukocyte elastase (HLE). With the exception of the antioxidant potential and the inhibition of HLE-action the lower molecular fraction possessed weaker activities and contained mainly hamamelitannin, catechin, and unidentified constituents (Erdelmeier et al, 1996).

An aqueous extract of the leaves of *Hamamelis* inhibited the growth of *Escherichia coli* (MIC 0.4 mg/ml), *Staphylococcus aureus* (MIC 0.4 mg/ml), *Bacillus subtilis* (MIC 1.1mg/ml) and *Enterococcus faecalis* (MIC 3.0mg/ml). Aqueous extracts of the bark inhibited the growth of *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Enterococcus faecalis* (MIC for all 10.0 mg/ml) (WHO monograph on *Hamamelis*, 2004).

**Eucalyptus globulus**

Two products from eucalyptus are in medicinal and commercial use, essential oil and dried extract, both obtained from leaves of *Eucalyptus globulus* Labill. (Myrtaceae). There are several hundred species of eucalyptus, most of them native to Australia. Like the Tea Tree, preparations of *Eucalyptus* have been an important part of traditional medicine of Australia’s Aboriginal people for thousands of years. Eucalyptus essential oil is primarily produced from the leaves of the Blue Gum Eucalyptus (*Eucalyptus globulus*), although other species of eucalyptus are also used.

The primary component of eucalyptus essential oil is eucalyptol (1.8-cineol). Eucalyptol is a monoterpenene molecule and it constitutes up to 90% of eucalyptus essential oil. In pure form, eucalyptol is a clear, colorless liquid that has a strong camphor-like smell. In addition to eucalyptus oil, eucalyptol is found in the essential oil of many other plants, although usually at lower concentrations. Eucalyptol is volatile and flammable and has a lower boiling point than water. It is also toxic to most animals when ingested in high quantities. Secondary components of eucalyptus essential oil are alpha-pinene, limonene, globulol and terpinen-4-ol (the primary component of tea tree oil). Several of these secondary components are known to have antibacterial and anti-inflammatory properties, but most of the activity of eucalyptus oil is attributed to its primary component, eucalyptol. On the other hand, the *Eucalyptus globulus* extract is one of the best-selling products today, manufactured from leaves of Blue Gum Eucalyptus and standardized on 25% of total chlorogenic acid (http://www.herb-extract.com/plant-extract/563839.html).

In traditional medicine, eucalyptus leaves have been used to prepare compresses, poultices, teas, etc. Eucalyptus essential oil and eucalyptol are both used extensively in modern medicine. Eucalyptol is toxic to many types of bacteria and is one of the active ingredients in antibacterial mouthwashes. Eucalyptol also has anti-inflammatory and cough suppressant properties, and is an ingredient in many cough drops. Inhalation of eucalyptol vapors is an effective short term analgesic and decongestant. Eucalyptus oil is a natural insect repellent for pests like mosquitoes (although it attracts certain types of bees).

There is little direct research into the effectiveness of eucalyptus essential oil in the treatment of acne. However, it is certainly possible that eucalyptus essential oil would be helpful in treating acne because of its antibacterial and anti-inflammatory properties (Athikomkulchai et al., 2008; Takahashi et al., 2004). The essential oil of *E. globulus* has a strong antimicrobial activity, especially against *Streptococcus pyogenes*, *Escherichia coli*, *Candida albicans*, *Staphylococcus aureus*, *Acinetobacter baumannii*, and *Klebsiella pneumonia* (Ghalem and Mohamed, 2008; Tabanca et al., 2001).

Essential oils of *Eucalyptus* species produced anti-inflammatory effects, demonstrated by inhibition of rat paw edema induced by carrageenan and dextran, neutrophil migration into rat peritoneal cavities induced by carrageenan, and vascular permeability induced by carrageenan and histamine (Silva et al., 2003).

Recent studies have shown that eucalyptus essential oil is effective against *P. acnes*, the primary bacterium in acne infections. It was reported that eucalyptus oil has similar antibacterial capabilities as benzoyl peroxide, a commonly used topical OTC medication for acne treatment (Athikomkulchai et al., 2008). Additionally, the anti-inflammatory properties of eucalyptus oil may also be beneficial in acne condition. However, like most topical acne treatments, topical application of eucalyptus do not necessarily deliver enough active ingredients to the site of infection. Even though eucalyptus oil is effective against *P. acnes in vitro*, there is no real evidence that topically applied eucalyptus oil penetrates effectively into the follicle and sebaceous glands and its efficacy in the treatment of acne is unproven, at this time. It is also important to notice that topical applications of high concentration eucalyptus oil can produce side effects (Darben et al., 1998).

The second important product from eucalyptus is leaf dried extract. It contains gallic acid, ellagic acid, glucosides of quercetin and kaempferol, tannin dimer, oenothein B, and a new galloctannin with structure 1,2,3,6-tetra-O-galloyl-beta-D-galactose (Amakura et al., 2009). The extract demonstrate strong antibacterial activity against *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Haemophilus influenzae* as well as on other gram positive (*C. pyogenes*, *S. aureus*, *S. faecalis*, *B. stearothermophilus*, *S. epidermis*, *B. cereus*, *B. polymyxia*, *B. anthracis*, *B. subtilis* and *C. sporogenes*) and gram negative bacteria (*K. pneumonia*, *P. aeruginosa*, *E. coli* and *P. fluorescens*) (Safari et al., 2006; Egwaikhide et al., 2008).

Eucalyptus bark extract represent also interesting nat-
Melaleuca alternifolia (Tea-tree)

Melaleuca alternifolia, Narrow-leaved Tea-tree, is a species of tree or tall shrub in the plant genus Melaleuca. Native to Australia, it occurs on the north coast and adjacent ranges of New South Wales. It grows along streams and on swampy flats, and is often the dominant species where it occurs. Characteristic of the myrtle family Myrtaceae, it is used to distil essential oil. It is the primary species for commercial production of Tea-tree oil (TTO, melaleuca oil), an essential oil with antibacterial (Carson et al., 2006) and antifungal activity (Hammer et al., 2003). More recently, the scientific community has confirmed that TTO has tremendous medicinal benefits and it is recognized as an excellent natural remedy for hundreds of bacterial and fungal skin ailments. Therefore it is used in a range of herbal medicine products and in cosmetic and toiletry products (deodorants, shampoos, soaps and lotions).

TTO is toxic if ingested in large amounts and if used topically in high concentrations may cause skin irritation (Hammer et al., 2006). No deaths have been reported.

TTO is a pale yellow color to nearly colorless and clear essential oil with a fresh camphoraceous odor. TTO should not be confused with tea oil, the sweet seasoning and cooking oil from pressed seeds of the tea plant Camellia sinensis (beverage tea), or the tea oil plant Camellia oleifera.

TTO is composed of terpene hydrocarbons, mainly monoterpenes, sesquiterpenes, and their associated alcohols. According to TTO required chemical composition, as per ISO 4730 (2004), its chemical composition comprises terpinen-4-ol (30-48%), γ-terpinene (10–28%), α-terpinene (5–13%), 1.8-cineole (0–15%), α-terpinolene (1.5–5%), α-terpineol (1.5–8%), α-pinene (1–6%) and p-cymene (0.5–8%) (Tea-tree oil, http://chemicalland21.com/lifescience/foco/TEA%20TREE%20OIL.htm).

Given the scope for batch-to-batch variation, it is fortunate that the composition of oil sold as TTO is regulated by an international standard for “Oil of Melaleuca—terpinen-4-ol type,” which sets maxima and/or minima for 14 components of the oil. Notably, the standard does not stipulate the species of Melaleuca from which the TTO must be sourced. Instead, it sets out physical and chemical criteria for the desired chemotype. Six varieties, or chemotypes, of M. alternifolia have been described, each producing oil with a distinct chemical composition. These include a terpinen-4-ol chemotype, a terpinolene chemotype, and four 1.8-cineole chemotypes. The terpinen-4-ol chemotype typically contains levels of terpinen-4-ol of between 30 to 40% and is the chemotype used in commercial TTO production (Homer et al., 2000). Despite the inherent variability of commercial TTO, no obvious differences in its bioactivity either in vitro or in vivo have been noted so far. The components specified by the international standard were selected for a variety of reasons, including provenance verification and biological activity. For example, with provenance, the inclusion of the minor components sabine, globulol, and viridiflorol is potentially helpful, since it may render the formulation of artificial oil from individual components difficult or economically untenable. With biological activity, the antimicrobial activity of TTO is attributed mainly to terpinen-4-ol, a major component of the oil. Consequently, to optimize antimicrobial activity, a lower limit of 30% and no upper limit were set for terpinen-4-ol content. Conversely, an upper limit of 15% and no lower limit were set for 1.8-cineole, although the rationale for this may not have been entirely sound. For many years cineole was erroneously considered to be a skin and mucous membrane irritant, fuelling efforts to minimize its level in TTO. This reputation was based on historical anecdotal evidence and uncorroborated statements (Williams and Home, 1988; Williams et al., 1990; Williams et al., 1993), and repetition of this suggestion appears to have consolidated the myth.

Recent data, do not indicate that 1.8-cineole is an irritant. Although minimization of 1.8-cineole content on the basis of reducing adverse reactions is not warranted, it remains an important consideration since 1.8-cineole levels are usually inversely proportional to the levels of terpinen-4-ol (Brophy et al., 1989), one of the main antimicrobial components of TTO (Carson and Riley, 1995; Raman et al., 1995; Carson et al., 2006).

Antimicrobial activity of TTO has received the most attention. The few earlier reports of the antibacterial activity of the TTO (Walsh and Longstaff, 1987; Low et al., 1974) have been reviewed (Carson et al., 1993; Christoph et al., 2000; Lis-Balchin et al., 2000; Messager et al., 2005) and in general previously obtained results were confirmed.

In the last two decades, many reports describing the antimicrobial activity of TTO appeared in the scientific literature. Although there was still a degree of discrepancy between the methods used in the different studies, the MICs reported were often relatively similar. A broad range of bacteria have now been tested for their susceptibilities to TTO. While most bacteria are susceptible to TTO at concentrations of 1.0% or less, MICs in excess of 2% have been reported for organisms such as commensal skin staphylococci and micrococci, Enterococcus faecalis, and Pseudomonas aeruginosa (Hammer et al., 1996). Few researchers published lower value of MIC (0.25%) of TTO for Enterobacter aerogenes, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Salmonella choleraesuis, Shigellex flexneri, Bacillus subtilis, Listeria monocytogenes, Staphylococcus aureus, S. saprophyticus, and S. xylosus (Harkenthal et al., 1999).

TTO is for the most part bactericidal in nature, although it may be bacteriostatic at lower concentrations. The activ-
ity of TTO against antibiotic-resistant bacteria has attract-
ed considerable interest, with methicillin-resistant *Staph-
ylococcus aureus* (MRSA) receiving the most attention thus far. Several groups have evaluated the activity of TTO against MRSA, beginning with Carson et al. (1995), who examined 64 MRSA isolates from Australia and the United Kingdom, including 33 mupirocin-resistant isolates. The MICs and minimal bactericidal concentrations (MBCs) for the Australian isolates were 0.25% and 0.5%, respectively, while those for the United Kingdom isolates were 0.312% and 0.625%, respectively (Carson et al., 2006). Using a TLC-bioautographique technique Raman et al. (1995) investigated the antibacterial activity of TTO and isolated ter-
pine-4-ol, alpha-terpineol and alpha-pinene, against *Staphylo-
coccus aureus*, *S. epidermidis* and particularly against *P. acnae*. The obtained results supported the use of TTO in acne treatment, demonstrating that terpinene-4-ol was not the sole active constituent of the oil. Among antibacterial, *in vitro* investigation of TTO against dermatophytes and filamentous fungi shown inhibitory and fungicidal activity (Hammer et al., 2002).

The mechanism of action of TTO against bacteria has now been partly elucidated. Prior to the availability of data, assumptions about its mechanism of action were made on the basis of its hydrocarbon structure and attendant lipophi-
licity. Since hydrocarbons partition preferentially into bio-
logical membranes and disrupt their vital functions, TTO and its components were also presumed to behave in this manner. This premise is further supported by data showing that TTO permeabilizes model liposomal systems. In previous work with hydrocarbons not found in TTO and with terpenes found at low concentrations in TTO, lysis and the loss of membrane integrity and function manifested by the leakage of ions and the inhibition of respiration were demonstra-
ston. Treatment with TTO sensitized *S. aureus* cells to sodium chloride and produced morphological changes apparent under electron microscopy. Furthermore, no cy-
toplasmic membrane damage could be detected using the lactate dehydrogenase release assay, and only modest up-
take of propidium iodide was observed after treatment with TTO (Carson et al., 2006; Cox et al., 2000).

In parallel with the characterization of the *in vitro* anti-
microbial activity of TTO, the clinical efficacy of the oil has also been the subject of investigation. One of the first rigorous clinical studies assessed the efficacy of 5% TTO in the treatment of acne by comparing it to 5% ben-
zoyl peroxide (Bassett et al., 1990). The study found that both treatments reduced the numbers of inflamed lesions, although benzoyl peroxide performed significantly bet-
ter than TTO. The benzoyl peroxide group showed signif-
ificantly less oiliness than the TTO group, whereas the TTO group showed significantly less scaling, pruritis, and dry-
ness. Significantly fewer overall side effects were report-
ed by the TTO group (27 of 61 patients) than by the benzo-
yl peroxide group (50 of 63 patients). Few years ago, En-
shaieh et al. (2007) confirmed the efficacy of 5% topical TTO gel in treatment of moderate *acne vulgaris* in a ran-
domized, double-blind placebo-controlled study. The effi-
cacy of TTO in dental applications, for the eradication of MRSA carriage, in the possibility of using TTO in hand-
wash formulations for use in hospital or health care set-
tings and as a mouthwash in the treatment of oropharynge-
al candidiasis, has been also evaluated in numerous clinical 

Numerous recent studies support the anti-inflammatory activity of TTO. Research studies performed over the last decade have demonstrated that TTO affects a range of immune responses, both *in vitro* and *in vivo*. For example, the water-soluble components of TTO can inhibit the lipopoly-
saccharide-induced production of the inflammatory medi-
ators tumor necrosis factor alpha (TNF-α), interleukin-
1β (IL-1β), and IL-10 by human peripheral blood mono-
cytes by approximately 50% and that of prostaglandin E,
by about 30% after 40 h (Hart et al., 2000). Further exam-
ination of the water-soluble fraction of TTO identified ter-
pine-4-ol, alpha-terpineol, and 1.8-cineole as the main com-
ponents, but of these, only terpinen-4-ol was able to dimin-
ish the production of TNF-α, IL-1β, IL-8, IL-10, and pro-
taglandin E, by lipopolysaccharide-activated monocytes. The water-soluble fraction of TTO, terpinen-4-ol, and alpha-terpineol also suppressed superoxide production by ago-
nist-stimulated monocytes but not neutrophils (Brand et al., 2001). In contrast, similar work found that TTO decreases the production of reactive oxygen species by both stimul-
ed neutrophils and monocytes and that it also stimulates the production of reactive oxygen species by nonprimed neu-

trophils and monocytes (Caldefie-Chézet et al., 2004). Hu-
man studies on histamine-induced wheal and flare provid-
ed further evidence to support the clinical use of the *in vitro* and animal data, with the topical application of neat TTO significantly re-
ducing mean wheal volume but not mean flare area (Koh et al., 2002). Work has now shown that terpinen-4-ol, but not 1.8-cineole or alpha-terpineol, modulates the vasodilation and plasma extravasation associated with histamine-induced in-
flammation in humans (Khalil et al., 2004).

**Ocimum sanctum** (Holy basil)

In traditional systems of medicine, different parts (leaves, stem, flower, root, seeds and even whole plant) of *Ocimum sanctum* Linn. (known as Tulsi in Hindi), a small herb seen throughout India, have been recommended for the treatment of bronchitis, bronchial asthma, malaria, di-
arrhea, dysentery, skin diseases, arthritis, painful eye dis-
eses, chronic fever, insect bite etc. The *Ocimum sanctum* has also been suggested to possess antifertility, anti-
cancer, anti-diabetic, anti-fungal, antimicrobial, hepatopro-
tective, cardioprotective, anti-oxidative, anti-vasomod-ic, adaptogenic and diaphoretic actions (Mondal et al., 2009; Singh et al., 2007).

Eugenol (1-hydroxy-2-methoxy-4-allylanisole), the active constituent present in *Ocimum sanctum*, has been
found to be largely responsible for the therapeutic potentials of the plant. Although because of its great therapeutic potentials and wide occurrence in India the practitioners of traditional systems of medicine have been using *Ocimum sanctum* for curing various ailments. A rational approach to this traditional medical practice with modern system of medicine is, however, not much available (Prakash and Gupta, 2005).

Chemical composition of *O. sanctum* means presence of volatile oil (0.4-0.8%) containing chiefly eugenol app. 21% and β-caryophyllene 37% (eugenol content reaches maximum in spring and minimum in autumn). A number of sesquiterpenes and monoterpens such as bornyl acetate, β-elemene, methylcyclogeraniol, nerol, β-pinene, camphene, α-pinene etc. are also present as constituents of the oil. Besides, triterpene component ursolic acid, sterols (campesteryl, cholesterol, stigmasterol, β-sitosterol) and methyl esters of common fatty acids are also key constituents of the plant oil (*Ocimum sanctum*, http://101herbs.com/ocimum_sanctum.html).

From fresh leaves and stems of *O. sanctum* and further purification of the obtained extract, the few phenolic compounds were isolated: cirsilinole, cisinarin, isothymusin, isothymonin, apigenin, and rosmarinic acid, and appreciable quantities of eugenol (Kelm et al., 2000).

Gupta et al. (2007) isolated three new compounds, ocimumosides A and B and ocimarin (Fig. 6), were isolated from an extract of the leaves of holy basil, together with eight known substances, apigenin, apigenin-7-O-β-d-glucopyranoside, apigenin-7-O-β-d-glucuronic acid, apigenin-7-O-β-d-glucuronic acid 6″-methyl ester, luteolin-7-O-β-d-glucuronic acid 6″-methyl ester, luteolin-7-O-β-d-glucopyranoside, luteolin-5-O-β-d-glucopyranoside, and 4-allyl-1-O-β-d-glucopropylsyl-2-hydroxybenzene, and two cerebrosides.

In order to establish the therapeutic uses of *O. sanctum* in modern medicine, in last few decades several Indian scientists and researchers have studied the pharmacological effects of steam distilled, petroleum ether and benzene extracts of various parts of the plant and eugenol on immune system, reproductive system, central nervous system, cardiovascular system, gastric system, urinary system and blood biochemistry and have described the therapeutic significance of *O. sanctum* in management of various ailments. These pharmacological studies have established a scientific basis for therapeutic uses of this plant (Prakash and Gupta, 2005).

The most studies on biological effects are based on antimicrobial activity of *O. sanctum* essential oil (Dey and Choudhari, 1984; Mondal et al., 2007). The essential oil of *O. sanctum* has been effective against gram-positive and gram-negative bacteria and the properties were comparable with the effectiveness of clove oil. It also exhibited significant antimicrobial activities against some of the clinical isolates and multi-drug resistant Neisseria gonorrhoeae (Mondal et al., 2009). A comparative investigation has shown that the oil of sweet basil (*O. basilicum*) was even more effective against the *P. acnes*, in comparison to the oil of holy basil (*O. sanctum*), but both oils could be recommended for use in micro-emulsion formulations for acne skin care (Viyoch et al., 2006).

The aqueous and methanolic suspension of *O. sanctum* has shown to inhibit acute as well as chronic inflammation in rats. The test was conducted by carrageenan induced paw edema, croton oil induced granuloma and exudates, at a dose of 500 mg/kg, bw/day (Godhwani et al., 1987). The oils extracted from fresh leaves (essential oil) and seeds (fixed oil) of *O. sanctum* have shown anti-inflammatory effects on experimental animals hind paw edema induced by carrageenan, serotonin, histamine and prostaglandin-E-2. These experimental rats were administered with essential oil (200 mg/kg, bw), and fixed oil (0.1ml/kg, bw) before injection of phlogistic agents and was compared with standard drug flurbiprofen. It was noted that extracts could significantly reduce the edema when compared with the saline treated control. However, its effect was less than the standard drug (Singh and Agarwal, 1991).

The mechanism of action of the anti-inflammatory effects of *O. sanctum* could be the cyclo-oxygenase and lipoxygenase pathways (Singh et al., 1996; Singh and Majumdar, 1995; 1997). In order to compare the anti-inflammatory effects of fixed oils of various species of *Ocimum* vs. *O. sanctum, O. basilicum, O. americanum*, which possess varying proportions of unsaturated fatty acids (par-

![Fig. 6. Structures of ocimumosides A (1) and B (2) and ocimarin (Gupta et al., 2007).](image-url)
particularly linolenic acid) showed different response against phlogistic agent induced paw edema. *Ocimum basilicum* possess highest percentage of linolenic acid (21%) and offered maximum inhibition of paw edema (72.42%). *O. Sanctum* fixed oil containing 16.63% linolenic acid provided 68.97% inhibition while *O. americanum* offered least paw edema inhibition (Singh, 1998). Fixed oil of *O. sanctum* can inhibit enhanced vascular permeability and leukocyte migration as evidenced by carrageenan induced inflammatory stimulus (Singh et al., 1996). Extract of seeds from three plants including *Ocimum sanctum* have been studied for anti-inflammatory effects of carrageenan, leukotrine and arachidonic acid induced paw edema in rats. *Ocimum sanctum* seed oil showed maximum percentage inhibition of leukotrine induced paw edema (Singh et al., 2008). According to Prakash and Gupta (2005), eugenol, active constituent of the *O. sanctum* essential oil, has been found as largely responsible for the therapeutic potentials of the plant. Anti-inflammatory activity of the eugenol isolated from the essential oil of *O. sanctum* was studied in Wistar rats by using carrageenan induced Hind paw edema method (Thakur and Pitre, 2009). The isolated eugenol and anti-inflammatory drug paracetamol (positive control) exhibited significant activity when compared with carrageenan control.

**Calendula officinalis** (Marigold)

*Calendula officinalis*, or (pot) marigold, is a common garden plant belonging to the Asteraceae family. Native to Southern Europe, *Calendula* grows up to 60 cm in height and produces large yellow or orange flowers. Like many other members of the Asteraceae family, which include daisies, arnica, chamomile, and yarrow, calendula is now cultivated throughout the world and is valued for its culinary and medicinal uses. The flowers are the part of the herb used medicinally (mainly because of its antibacterial, anti-inflammatory and antioxidant properties) either in

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Fig. 7. Saponins of *Calendula officinalis* (http://www.medicinescomplete.com/mc/herbals/current/images/HrbcalendulaC001_default.png).
the form of infusions, tinctures, liquid extracts, creams or ointments, or in one of a number of skin and hair products available as OTC or cosmetics.

A number of phytochemical studies have demonstrated the presence of several classes of chemical compounds in flowers or in other organs of marigold, the main ones being terpenoids, flavonoids, coumarines, quinones, volatile oil, carotenoids and amino acids (Muley et al., 2009). Various terpenoids and sterols have been reported from the petroleum ether extract of Calendula officinalis flowers such as: sitosterols, stigmasterols, diesters and monoesters of taraxasterol, lupeol, erythrodial, ursadiol, faradiol, arnidiol, calenduladial, oleanolic acid saponins (calenduloside AH) and oleanane triterpene glycoside (calendulaglycosides) (Fig. 7). One new triterpene ester of olanane series isolated from the flowers was cornulacic acid acetate (Naved et al., 2005).

Various flavonoids have been isolated from the ethanol extract of the inflorescence of C. officinalis. They include quercetin, isorhamnetin, isoquercetin, narcissin, calendiflaside, calendoflavoside, calendoflavobioside, rutin, isoquercitrin neohesperidoside, and different glycosides of isorhamnetin and quercetin (Muley et al., 2009; Vidal-Oliver et al., 1989). Different quinones, volatile oil, carotenoids, carbohydrates, lipids and other constituents were also identified in marigold flowers (Muley et al., 2009).

For centuries, marigold flowers have been used to treat a number of clinical conditions, specifically, different dermatological disorders. Whilst the many chemical constituents within marigold and the numerous actions of the plant suggest that marigold may be effective in treating a myriad of complaints. However, there is currently insufficient clinical evidence to support the use of pot marigold in conditions other than cutaneous lesions.

Marigold is considered a mainstay in alternative medicine for the treatment of inflammation, to speed wound healing and as an antiseptic. Available in topical herbal forms and as a homeopathic preparation, the anti-inflammatory (Braga et al., 2009; Chandran and Kuttan, 2008; Chandran et al., 2009; Della Loggia et al., 1994; Ukiya et al., 2006) and anti-bacterial (Lauk et al., 2003) properties of marigold may be helpful for treating dermatological conditions including acne (Muley et al., 2009).

Calendula officinalis flower extract have been proved for possessing significant anti-inflammatory activity against carragenan and dextran-induced acute paw edema. In recent study conducted on flower extracts to find out mechanism involved in this, it was found that TNF-alpha production by macrophage culture treated with lipopolysaccharide was inhibited by C. officinalis extract. C. officinalis also contains flavonoids, which accounts for its anti-inflammatory impact (Preethi et al., 2009). Different hydroalcoholic extracts of marigold possesses proven antimicrobial, antifungal and antiviral properties against Staphylococcus aureus and Streptococcus fecalis Prophyromonas gingivalis, Fusobacterium nucleatum, Capnocytophaga gingivalis, Veillonella parvula, Eikenella corrodens, Pseudotreptococcus micros and Actinomyces odontolyticus, Staphylococcus aureus, Sarcina lutea, Escherichia coli, Klebsiella pneumonia and Candida monosa on one hand, and on the other hand, the actiology of acne.

Calendula officinalis is available in a number of ointment, cream and salve preparations in a variety of strengths. Calendula oils and infusions are also widely available in health-food stores and through online sources. Calendula officinalis is also commonly available in tea and liquid tinctures which can be applied directly to the acne prone areas of the skin.

Conclusion

Much disparate and introductory research exists on the effects of herbs on multiple aspects of acne. A comprehensive approach combining multiple herbs as well as lifestyle and dietary changes has helped people with acne in preliminary clinical trials. The continued resistance of mainstream dermatology to the possibility of this approach does not optimally serve patients who might be significantly helped by natural therapies. There are sufficient pilot data to warrant larger trials on various herbal medicines in isolation and combined with each other and other natural therapies. The data are also sufficient to support a recommendation for use of these herbs in clinical practice. Overall, herbal medicine has much to offer to improve our ability to deal with the complex issues acne presents.

However, an appropriate delivery system should be developed to impart their efficacies in addition to the standardization of these herbs. Furthermore, an optimized and effective dose should be evaluated prior to the development of preparations in order to avoid irritation or allergy in subjects with hypersensitive skin. Strict quality control will ensure their safety and efficacy. In addition, combination treatment should be conducted as it was found to be more effective than the application of a single product with regard to synergistic effects on the pathogenesis of acne.

References


Макед. фарм. билт., 55 (1, 2) 3 - 22 (2009)
Med. 60 (6), 516-520.


compounds from Ocimum sanctum Linn. Phytomedicine 7(1), 7–13.


Maced. pharm. bull., 55 (1, 2) 3 - 22 (2009)

Marija Glavas Dodov and Svetlana Kulevanova

Antimicrobial activities of essential oils obtained from fresh and dried leaves of Ocimum sanctum (L.) against enteric bacteria and yeast. Acta Hort. (ISHS) 756, 267-270.


A review of phytotherapy of acne vulgaris


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Резиме

Фитотерапија на Acne vulgaris

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Ключни зборови: Acne vulgaris, третман на акни, фитотерапија на акни

Acne vulgaris (акне) е една од најчестите дерматози во современото општество. Акне е хронична полиморфна болест на пилосебеалините структури на кожата, што вклучува абио нормалности во продукцијата на себум и се карактеризира со појава на инфламаторни (папули, пустули, нодуси) и неинфламаторни (отворени и затворени комедони) лезии. Propionibacterium acnes и Staphylococcus epidermidis се значајни фактори во патогенезата на инфламаторните облици на акни, иако акне не претставува бактериска инфекција.

Денес на пазарот се присутни голем број лекови и козметички производи за третман на акни, при што се прифатени четири основни принципи: елиминирање на алтерираниот начин на кератини за цијанфоликулот, намалување на интрафоликуларна-та популација на Propionibacterium acnes или генерирањето на екстрацелуларни инфламаторни агенси и намалување на секрецијата на себум.

Третманот на акни вклучува локална и/или системска терапија, директна интралезиона терапија со кортикостероиди, фитотерапија и нивни комбинации. При локалната терапија најчесто се користат бензоил пероксид, локални антибиотици и ретиноидна киселина. Системската терапија опфаќа примената на антибиптици и орални ретиноиди. Изборот на третманот зависи од стадиумот на болеста, но истиот често е проследен со одредени несакани ефекти.

Во последните години, примената на хербалните преработки во третманот на акни добива секундарна научна потврда и се смета како ефикасна алтернатива на конвенционалната терапија.

Целта на овој труд е да даде сеопфатен преглед, базиран на научни докази, на растителните суровини и на фитопрепаратите со потврдена клиничка ефикасност што се користат во третманот на акни.
Chemical characterization and antioxidant activity of *Eryngium campestre* L., Apiaceae from Kosovo

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**Abstract**

This study is outlined to define the chemical composition and *in vitro* antioxidant activity of the extracts of aerial part and root of *Eryngium campestre* L. (Apiaceae) from Kosovo. Analysis of the chemical composition include determination of total ash, ash insoluble in hydrochloric acid, loss on drying and the content of water extract, as well as determination of flavonoids in aerial part and hemolytic activity of the root. The mineral composition (Zn, Fe, Cu, Mn, Ni, K, Co, Pb, Cd and Cr) in aerial parts and root has been studied using atomic absorption spectroscopy (AAS and ETAAS). Different part of *E. campestre* accumulate different amounts of investigated minerals. Antioxidant activity was determined by four various testing systems: DPPH assay, inhibition of production of hydroxyl radical, β-carotene-bleaching assay, and inhibition of lipid peroxidation (TBA test). In DPPH system, ethanol extract of root of *E. campestre* exhibited high radical-scavenging activity (IC₅₀ = 0.72 mg ml⁻¹) compared to the extract of the aerial part (IC₅₀ = 1.14 mg ml⁻¹). On the other hand, aerial part ethanol extract has exhibited stronger inhibition capacity on the production of hydroxyl radical in deoxyribose system than the root extract (50% and 45%, respectively). However, both ethanol extracts of *E. campestre* exhibited low antioxidant activity in β-carotene-bleaching assay as well as, low capacity for inhibition of spontaneous lipid peroxidation in rat liver homogenate.

**Key words:** *Eryngium campestre*, flavonoids, mineral content, DPPH assay, antioxidant activity, β-carotene-bleaching test, TBA test.

**Introduction**

*Eryngium campestre* L. (Apiaceae) (field eryngo) is perennial plant, spread in Spain, France, Germany, Balkan Peninsula and other scattered localities in Europe, and in Africa and Asia as well (Ingram, 2006). It has been used in folk medicine as an infusion to treat cough, whooping cough, urinary infections, disturbed functions of kidney, increased urine secretion, eliminating out stones and sand from kidney and bladder, against water retaining and other conditions on urinary tract, for regulation of the function of prostate. The root of eryngo is known as antispasmodic, aromatic, diaphoretic, diuretic, expectorant, galactofuge and stimulant (Petkov, 1982). It promotes free expectoration and is very useful in the treatment of debility attendant on coughs of chronic standing in the advanced stages of pulmonary consumption. There are none known hazards of *Eryngium campestre*, even more young shoots and roots are edible parts of the plant which can be cooked and used as an asparagus substitute and as an easily digested vegetable (www.gardenzone.info).

The presence of bioactive secondary metabolites including saponins, phenolic acids, flavonoids, coumarins, essential oil, is considered to determine pharmacological
activities of the plants of genus *Eryngium*, as well. Considering phenolics, flavonoids are the most investigated compounds. The literature data show that *Eryngium campestre* contain glycosides of kaempferol, isorhamnetin, luteolin and quercetin (Karting and Wolf, 1993; Nebija et al. 2006) and flavanolacetyl glycosides (Hohman et al. 1997) while the other representatives of the genus such as *Eryngium planum* kaempferol and its glycosides (Stecka-Paszkieiew, 1983, Zarneck et al. 1977), *E. maritimum* contains glycosides of kaempferol, isoquercetin and astragalin (Hiller et al. 1981) and *E. creticum* glycosides of quercetin (Al-Khail, 1994). *E. campestre* contains coumarins (Erdelmeier and Sticher, 1985), D-mannitol (Asenov and Grevrenova, 1991), cyclohexanone and cyclohexadienone glycosides (Erdelmeier and Sticher, 1986). Similar components were identified in other species of *Eryngium*, for example acetilenes in *E. creticum* (El-Gamal et al. 1978) and coumarins in *E. ilicifolium* (Pinar and Galan, 1985). However, the most important class of secondary metabolites investigated in root of *Eryngium campestre* and other species of *Eryngium* were saponins. Thus, Kartal et al. were the first that had reported presence of two baringenol saponins in roots of *E. campestre*. These structures were elucidated by 2D NMR and mass spectrometry (Kartal et al. 2005). Five other saponins also glycosides of baringenol were reported one year latter (Kartal et al. 2006). Hiller at al. reported presence of eryngium saponins A, A1, A2 and B in root of *E. planum* (Hiller et al. 1972), then saponins of betulinic and oleanolic acids in *E. bromelifolium* (Hiller et al. 1974; 1976; 1978) and similar saponins in *E. maritimum* (Hiller et al. 1976). According to this, saponins were considered as the class of components responsible for anti-inflammatory effects of the root extract from *E. campestre*, but also for root and herb extracts of *E. maritimum*, *E. kotschyi*, *E. creticum* (Kupeli et al. 2006; Lisciani et al. 1984). Extracts of *E. creticum* could act beneficially against the hemolytic activities of snake and scorpion venoms (Alkohafi et al. 1997), while *E. foetidum* leaf extract acts as anticonvulsant, anti-inflammatory and analgesic agent (Simon and Singh, 1986; Saenz et al. 1998). Representatives of *Eryngium* species contain significant amounts of volatile oils and their oil composition and possible biological activity were investigated as well (Capeta nos et al. 2007; Lerclercq et al. 1992; Pala-Paul et al. 2005; Pino et al. 1997a, 1997b; Wong et al. 1994). Finally, lypo phenic extracts of species of genus *Eryngium* contain different phytosterols which are considered as important constituents for topical anti-inflammatory activity on acute and chronic inflammation models (Garcia et al. 1999).

*Eryngium campestre* is widely spread throughout the territory of Kosovo. Until now there is no data of chemical composition and biological activity of *Eryngii herba* or *Eryngii radix* originated from Kosovo. Having in mind all previously mentioned, the aim of the present research is determination of the chemical composition and possible antioxidant activity of different parts of *Eryngium campestre* from Kosovo.

**Experimental**

**Plant material**

The samples of the aerial parts in full blossom (*Eryngii herba*) were collected during summer in 2002 and 2003 on three different locations in Kosovo: 1) Prishtina, 2) Poduevo and 3) Lipjan. The roots (*Eryngii radix*) were collected at the same locations in autumn 2002 and 2003, as well. The samples were labeled with following marks for samples collected from 1) Prishtina, 2) Poduevo and 3) Lipjan, respectively:

- EH1/02, EH2/02 and EH3/02 for aerial plant material collected in 2002,
- EH1/03, EH2/03 and EH3/03 for aerial plant material collected in 2003,
- ER1/02, ER2/02 and ER3/02 for the roots collected in 2002, and
- ER1/03, ER2/03 and ER3/03 for the roots collected in 2003.

Herbarium voucher specimens with the same marks were deposited at the Institute of Botany, Section Pharmacy at the Faculty of Medicine in Prishtina, Kosovo. All samples were left to air dry and then put in a paper bags and stored at cool, dry and dark place, until analysis.

**Determination of basic chemical parameters**

The contents of total ash and ash insoluble in hydrochloric acid as well as the loss on drying were determined using methods described in European Pharmacopeia (Ph. Eur. 4). The content of total water extractive was determined by method in Ph. Jug. IV.

**Determination of flavonoids**

The content of flavonoids was determined in the samples of *Eryngii herba* by UV/Vis spectrophotometry, using AlCl₃ as complexation reagent and method for determination of flavonoids described in Ph. Eur. 4. The spectrophotometer analysis was carried out in an Ultraviolet visible equipment (Perkin Elmer Lambda 16) at 425 nm. The results were expressed in percentage of flavonoids, calculated as quercetin, from the average of six determinations, using the calibration curve of quercetin (concentration range 0-100 µg.ml⁻¹; \( y = 0.0103x + 0.0066, R^2 = 0.9993 \)).

**Determination of hemolytic activity**

Haemolytic activity (expressed as HA) was evaluated for the root of *Eryngium campestre* by the method given in the Pharmacopoeia Jugoslavica (Ph. Jug. 4) using the Saponin-standard (HA = 30000) as the reference substance. The value of hemolytic activity (SU/g) was calculated using following equation:

\[
    HA = \frac{c \cdot a}{b \cdot d}
\]

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where: \( a \) = minimum volume (ml) of saponin-standard solution that provoke total hemolysis; \( b \) = minimum volume (ml) of extract that provoke total hemolysis; \( c \) = mg of saponin-standard in 100 ml standard solution; \( d \) = g of plant material in 100 ml extract.

**Determination of mineral content**

**Sample preparation.** Microwave-assisted digestion in a Milestone Touch Control microwave digestion system was used for mineralization purposes. To 0.5 g herbal material 2 ml of conc. HNO\(_3\) and 1 ml of 30% H\(_2\)O\(_2\) were added and the mixture was subjected to microwave digestion with following program:

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature/°C</th>
<th>Duration/min</th>
<th>Power/W</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>180</td>
<td>10</td>
<td>800</td>
</tr>
<tr>
<td>2</td>
<td>180</td>
<td>15</td>
<td>800</td>
</tr>
</tbody>
</table>

After cooling, the obtained solution was transferred into 50 ml volumetric flask and filled up with 4% HNO\(_3\). With each set of digested samples, a blank sample was run through the digestion procedure.

**Instrumentation condition.** Macrolelements were determined by atomic absorption spectrometry (AAS). A Varian SpectrAA 640Z Zeeman electrothermal atomic absorption spectrophotometer with a Varian PSD-100 Autosampler and Varian SpectrAA 880 with deuterium correction (for flame determination) were used. Hollow cathode lamps were used as a source. Operating conditions for the determination of Pb, Co, Ni, Cr, Fe, Mn, Na, K, Cd and Zn are given in Tables 1 and 2.

**Determination of the radical scavenging and antioxidant activity**

**Sample preparation.** Dried powdered plant material (10 g) was extracted by continual mixing in 100 ml

### Table 1. Instrumental parameters for determination of Na, K, Ni, Fe, Mn, Cu and Zn by flame AAS

<table>
<thead>
<tr>
<th>Element</th>
<th>Wavelength, nm</th>
<th>Slit, nm</th>
<th>Lamp current, mA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>589.0</td>
<td>0.2</td>
<td>5</td>
</tr>
<tr>
<td>K</td>
<td>766.5</td>
<td>1.0</td>
<td>5</td>
</tr>
<tr>
<td>Ni</td>
<td>232.0</td>
<td>0.2</td>
<td>4</td>
</tr>
<tr>
<td>Fe</td>
<td>248.3</td>
<td>0.2</td>
<td>5</td>
</tr>
<tr>
<td>Mn</td>
<td>279.5</td>
<td>0.2</td>
<td>5</td>
</tr>
<tr>
<td>Cu</td>
<td>324.8</td>
<td>0.2</td>
<td>5</td>
</tr>
<tr>
<td>Zn</td>
<td>213.9</td>
<td>1.0</td>
<td>5</td>
</tr>
<tr>
<td>Gas mixture</td>
<td>Acetylene/air</td>
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</tbody>
</table>

### Table 2. Optimal parameters for Co, Ni, Pb, Cd and Cr determination by Zeeman ETAAS

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Co</th>
<th>Ni</th>
<th>Pb</th>
<th>Cd</th>
<th>Cr</th>
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<tbody>
<tr>
<td>Wavelength, nm</td>
<td>242.5</td>
<td>232.0</td>
<td>283.3</td>
<td>228.8</td>
<td>357.9</td>
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<tr>
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<td>0.5</td>
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<td>0.5</td>
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<tr>
<td>Lamp current, mA</td>
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<td>4.0</td>
<td>5.0</td>
<td>4.0</td>
<td>7.0</td>
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<tr>
<td>Calibration mode</td>
<td>Absorbance, peak height</td>
<td>Argon</td>
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<td></td>
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<td>GAS DRYING</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Temperature (°C)</td>
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<td>120</td>
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<td>120</td>
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<tr>
<td>Ramp time (s)</td>
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<td>45</td>
<td>45</td>
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</tr>
<tr>
<td>Hold time (s)</td>
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<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
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<td></td>
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</tr>
<tr>
<td>Temperature (°C)</td>
<td>750</td>
<td>800</td>
<td>400</td>
<td>250</td>
<td>100</td>
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<tr>
<td>Ramp time (s)</td>
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<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
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<td>32</td>
<td>2</td>
<td>3</td>
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<td>2400</td>
<td>2100</td>
<td>1800</td>
<td>2500</td>
</tr>
<tr>
<td>Ramp time (s)</td>
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<td>1</td>
<td>0.7</td>
<td>1.2</td>
</tr>
<tr>
<td>Hold time (s)</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>CLEANING</td>
<td></td>
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</tr>
<tr>
<td>Temperature (°C)</td>
<td>2300</td>
<td>2400</td>
<td>2100</td>
<td>1800</td>
<td>2500</td>
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<tr>
<td>Hold time (s)</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

* Program for steps 1-3 is the same for all elements
ethanol:water (7:3, V/V), 24 h at room temperature. After filtration, filtrate was evaporated until dry. The residues were dissolved in 96% ethanol to obtained solution with concentration 0.1 g ml\(^{-1}\).

**Reagent and standards.** The standards of quercetin and BHA (butyl hydroxyl anisole) were purchased from Extrasynthese, Lyon, France. All other chemicals were of reagent grade and were used without further purification.

**Assesment of the free radical scavenging activity in 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) assay.** The antioxidant activity using the DPPH assay was assessed by the method of Tagashira and Ohtake (1998). A test sample solution (plant extract) (200 µl) was added to 4 ml of 100 mmol l\(^{-1}\) ethanolic DPPH. After vortexing, the mixture was incubated for 10 minutes at room temperature and the absorbance was measured at 517 nm. The differences in absorbance between a test sample and a control (ethanol) was considered as an activity. The activity was shown as IC\(_{50}\) value. Quercetin and BHA (100 mg ml\(^{-1}\) in ethanol) were used as reference substances. All values are shown as a mean value of the three measurements.

**Assesment of the hydroxyl radical scavenging activity.** Hydroxyl radical scavenging activity was carried out by measuring the competition between deoxyribose and the plant extracts for hydroxyl radicals generated from the Fe\(^{3+}\)/ascorbate/EDTA/H\(_2\)O\(_2\) system. The attack of the hydroxyl radical on deoxyribose leads to TBARS (thiobarbituric acid-reactive substances) formation (Halliwell and Gutteridge, 1981). The extracts were added to the reaction mixture containing 2.8 mmol l\(^{-1}\) deoxyribose, 100 mmol l\(^{-1}\) FeCl\(_3\), 104 mmol l\(^{-1}\) EDTA, 100 mmol l\(^{-1}\) ascorbic acid, 1 mmol l\(^{-1}\) H\(_2\)O\(_2\), and 230 mmol l\(^{-1}\) phosphate buffer (pH 7.4), making up a final volume of 1.0 ml. In the series of control experiments reference substances, such as quercetin and BHA (100 mg ml\(^{-1}\) in phosphate buffer-pH 7.4), were used instead of the extracts. The reaction mixture was incubated at 37 °C for 1 h. The formed TBARS were measured by the method given by Ohikawa et al. (1979). One ml of thio-barbituric acid TBA (1%) and 1.0 ml trichloroacetic acid (TCA 2.8%) were added to the tested tubes and were incubated at 100 °C for 20 min. After cooling, absorbance was measured at 532 nm against a blank containing deoxyribose and buffer. Reactions were carried out in triplicate.

**Assesment of antioxidant activity.** The antioxidant activity of the plant extracts was evaluated using β-carotene-bleaching assay (Wanasundara et al. 1994). A solution of β-carotene was prepared by dissolving 2.0 mg of β-carotene in 10 ml chloroform. One ml of this solution was pipetted into a round-bottom flask. When chloroform was rotary evaporated at 40 °C under vacuum, 20 mg of purified linoleic acid, 200 mg of Tween 40 emulsifier and 50 ml of distilled water were added to the flask with vigorous shaking. Aliquots (5 ml) of this emulsion were transferred into a series of tubes containing 2 mg of each plant extract or 2 mg of BHA (butylated hydroxyanisole) or 2 mg of quercetin, for comparison. Aliquots (5 ml) of emulsion without any further additions were used as control. As soon as the emulsion was added to each tube, the zero time absorbance was read at 470 nm. Subsequent absorbance readings were recorded at 10-min intervals by keeping the sample in a water bath at 50 °C until the visual color of β-carotene in the control sample had disappeared (about 120 min).

**Assesment of the capacity of inhibition of spontaneous lipid peroxidation (LP).** The quantitative measurement of lipid peroxidation was done by measuring the concentration of thiobarbituric acid reactive substances (TBARS) in liver homogenate using the method of Ohkawa et al. (1979). The amount of formed malondialdehyde (MDA) was quantitated by reaction with thiobarbituric acid and used as a measure of lipid peroxidation. The results were expressed as nmol MDA.mg\(^{-1}\) protein. The content of protein was determined according to the method of Bradford using bovine serum albumin as a standard (Bradford, 1976).

**Preparation of liver homogenate.** The rat livers were exposed, dissected free from extraneous tissues, rinsed with chilled 1.15% KCI solution (pH 7.0) and 50% homogenate was prepared in 0.15 mol l\(^{-1}\) sodium phosphate buffer (pH 7.0). The homogenate was centrifuged at 3500 g for 10 minutes at 4 °C and the supernatant was used for the estimation of lipid peroxidation level.

**Preparation of control:** to 0.5 ml of liver homogenate supernatant (LHS), 10 µl H\(_2\)O and 4.5 ml extractive solvent (10 ml 10 % HClO\(_4\) saturated with TBA and 30 ml 20% TCA) were added and heated on 95 °C for 20 minutes. After cooling, the mixture was centrifuged at 3500 g, 10 minute. Absorbance of transparent supernatant was measured on 532 nm.

**Preparation of test solution:** to 0.5 ml of liver homogenate supernatant (LHS), 10 µl of plant extract (or reference substance solution) and 4.5 ml extractive solvent were added and heated on 95 °C for 20 minutes. After cooling, the mixture was centrifuged at 3500 x g, 10 minute. Absorbance of transparent supernatant was measured on 532 nm.

The inhibition of lipid peroxidation (%) was calculated using following equation:

\[
I\% = \frac{A_c - A_s}{A_c} \times 100
\]

A\(_c\) - absorbance of control; A\(_s\) - absorbance in presence of extract (reference substance).

The amounts of formed malondialdehyde (nmol MDA mg protein\(^{-1}\)) were calculated using following equation:

\[
cMDA = \frac{A}{\varepsilon} \times \frac{V_p}{V_i} \times 10^{-3} \times R
\]

A - absorbance; \(\varepsilon\) - 156 000 dm\(^3\)/mol; \(V_p\) - volume before centrifugation; \(V_i\) - volume of LHS; R - dissolution rate.
Chemical characterization and antioxidant activity of *Eryngium campestre* L., Apiaceae from Kosovo

Results and discussion

Basic chemical parameters

The basic parameters important for assessment of quality of dried aerial part of *Eryngium campestre* from Kosovo (*Eryngii herba*) and roots (*Eryngii radix*) included determination of total ash, ash insoluble in hydrochloric acid and loss on drying (Table 3). The values of loss on drying ranged from 7.0 to 9.5% in *herba* and from 7.0-9.9% in *radix*. The amounts of total ash were lower in samples of *herba* (5.8-7.3%) in comparison to those of *radix* (9.6-13.3%). Samples of *Eryngii herba* had lower percentage of water extract (18.3-24.5%) in comparison to the samples of *Eryngii radix* (29.6-36.6%). Obtained results showed presence of larger amounts of possible active components in *Eryngii radix*. The amount of 8.41% total ash from the leaves of *E. foetidisima* are reported previously (Borah et al. 2009).

Flavonoid content

The results of determination of total flavonoid content in *Eryngii herba* are presented in Table 4. The percentages of total flavonoids (0.12 - 0.14%) were expressed as total quercetin. Compared with literature data, the contents of total flavonoids were lower than those previously reported by other researchers. Thus, in dried aerial parts of *E. platum, E. campestre* and *E. maritimum* from Romania, total of 0.32 – 0.56% of flavonoids expressed as rutin were found (Suciu et al. 2006).

Hemolytic activity

The results of the evaluation of hemolytic activity of *Eryngii radix* are presented in Table 5. Obtained values were lower in comparison to the HA of some saponin-containing plants such as *Saponaria officinalis* (radix) (50 SU/g), *Herniaria glabra* (herba) (30 SU/g) or *Primula ver-

### Table 3. The levels of basic chemical parameters for assessment of quality of *Eryngium herba* and *Eryngium radix*, *Eryngium campestre* from Kosovo (%)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Loss on drying</th>
<th>Ash</th>
<th>Ash insoluble in hydrochlorid acid</th>
<th>Water extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>EH1/02</td>
<td>7.5</td>
<td>6.6</td>
<td>1.9</td>
<td>24.2</td>
</tr>
<tr>
<td>EH1/03</td>
<td>7.0</td>
<td>5.8</td>
<td>2.0</td>
<td>22.5</td>
</tr>
<tr>
<td>EH2/02</td>
<td>7.7</td>
<td>7.3</td>
<td>1.7</td>
<td>18.8</td>
</tr>
<tr>
<td>EH2/03</td>
<td>9.1</td>
<td>5.8</td>
<td>2.3</td>
<td>21.1</td>
</tr>
<tr>
<td>EH3/02</td>
<td>7.7</td>
<td>5.8</td>
<td>1.5</td>
<td>21.8</td>
</tr>
<tr>
<td>EH3/03</td>
<td>9.5</td>
<td>5.8</td>
<td>1.8</td>
<td>18.3</td>
</tr>
<tr>
<td>ER1/02</td>
<td>8.9</td>
<td>10.7</td>
<td>1.9</td>
<td>36.6</td>
</tr>
<tr>
<td>ER1/03</td>
<td>9.8</td>
<td>11.9</td>
<td>3.0</td>
<td>33.6</td>
</tr>
<tr>
<td>ER2/02</td>
<td>7.0</td>
<td>13.0</td>
<td>2.5</td>
<td>31.6</td>
</tr>
<tr>
<td>ER2/03</td>
<td>9.0</td>
<td>12.0</td>
<td>3.6</td>
<td>29.6</td>
</tr>
<tr>
<td>ER3/02</td>
<td>9.9</td>
<td>13.3</td>
<td>4.8</td>
<td>34.7</td>
</tr>
<tr>
<td>ER3/03</td>
<td>9.3</td>
<td>9.6</td>
<td>5.3</td>
<td>33.8</td>
</tr>
</tbody>
</table>

EH – samples of *Eryngii herba*, ER – samples of *Eryngii radix*, n = 3.

### Table 4. The content of total flavonoids in *Eryngii herba*, *Eryngium campestre*

<table>
<thead>
<tr>
<th></th>
<th>EH1/02</th>
<th>EH2/02</th>
<th>EH3/02</th>
<th>EH1/03</th>
<th>EH2/03</th>
<th>EH3/03</th>
</tr>
</thead>
<tbody>
<tr>
<td>(%)±Sd</td>
<td>0.12±0.01</td>
<td>0.14±0.01</td>
<td>0.13±0.01</td>
<td>0.13±0.03</td>
<td>0.13±0.04</td>
<td>0.14±0.03</td>
</tr>
</tbody>
</table>

n = 3; Sd – standard deviation; EH – samples of *Eryngii herba*

### Table 5. The values of hemolytic activity of *Eryngii radix*, *Eryngium campestre* (saponin units per g plant material, SU/g)

<table>
<thead>
<tr>
<th></th>
<th>ER1/02</th>
<th>ER2/02</th>
<th>ER3/02</th>
<th>ER1/03</th>
<th>ER2/03</th>
<th>ER3/03</th>
</tr>
</thead>
<tbody>
<tr>
<td>HI</td>
<td>14.4</td>
<td>15.3</td>
<td>15.8</td>
<td>14.9</td>
<td>16.3</td>
<td>14.08</td>
</tr>
</tbody>
</table>

ER – samples of *Eryngii radix*, n = 3.

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is (radix) (120 SU/g), (Ph. Jug. 5). Data of hemolytic activity of Eryngii radix are not available in the literature. Among information about the presence of triterpene saponins in Eryngium campestre and other Eryngium species, data of chemical structure of saponins could be found (Kartal et al. 2005; 2006). Romanian researchers investigated the chemical composition of indigenous Eryngium species in Romania (E. planum, E. campestre and E. maritimum) and found that triterpene saponins (determined by gravimetric method) were presented in the aerial part of the plants, ranging from 3.7-10.1% (Suciu et al. 2006).

### Mineral content

Determination of mineral content included determination of eight microelements (Cd, Co, Cr, Cu, Fe, Mn, Pb and Zn) and two macroelements (Na and K). The results obtained for the samples of Eryngii herba and Eryngii radix are presented in Table 6. Obtained results differ a lot depending on the plant organ or the origin of the sample. In some cases the content of investigated minerals in Eryngii herba was relatively constant such as that of Zn (15.14-20.39 mg kg⁻¹), Cr (0.7-1.57 mg/kg) or Cu (8.17-11.17 mg kg⁻¹) while for the other minerals higher variability occurs (Fe from 69.93-196.77 mg kg⁻¹; Mn 31.46-47.68 mg kg⁻¹). The content of toxic elements was very low, from 0.17-1.33 mg kg⁻¹ and from 0.05-0.12 mg kg⁻¹ for Pb and Cd, respectively. The content of K in Eryngii herba was very high, from 1765.10-2538.08 mg kg⁻¹ while the content of Na ranged from 43.65-94.7 mg kg⁻¹.

Similar results were obtained for the content of minerals in Eryngii radix (Table 6). The content of Mn was almost the same as it was found in herba, from 25.19-41.19 mg kg⁻¹, the content of Zn ranged from 15.55-25.29 mg kg⁻¹, and for Cu from 9.16-12.45 mg kg⁻¹. Great variability in the content of Fe was occurred again and significantly larger amounts were found in Eryngii radix ranging from 198.9-325.7 mg kg⁻¹. The content of Na in Eryngii radix was higher than in Eryngii herba, ranging from 170.2-590.9 mg kg⁻¹, while the content of K in Eryngii radix was also very high, from 743.7-961.8 mg kg⁻¹ but significantly lower than in Eryngii herba. The content of toxic elements was very low, from 0.10-1.76 mg kg⁻¹ and from 0.04-0.16 mg kg⁻¹ for Pb and Cd, respectively.

Generally, Eryngii herba accumulates larger amounts of K while Eryngii radix accumulates larger amounts of Na and Fe. Comparing with the literature data, the leaves of E. billardieri contain higher amount of N, K, Ca and Mg and lower amount of P, S and Na than some common vegetation, while the content of Fe, Mn, Zn and Cu were at the same level (Turan et al. 2003). In leaves of E. foetidissima, 24.26 mg g⁻¹ Fe and 11.26 mg g⁻¹ K were found (Boarah et al. 2009).

### Radical scavenging and antioxidant activity

**DPPH free radical scavenging activity**

Free radical scavenging activity of Eryngium extracts was determined by comparing the activity with that of referent substances (quercetin and BHA), which possess known antioxidant potential. The values obtained in DPPH assay for plant extracts are shown together with that of reference substances (Table 7). The highest scavenging effect was obtained with quercitin (IC₅₀=0.06 mg ml⁻¹) and the lowest one with the root extract of E. campestre (IC₅₀=1.14 mg ml⁻¹). The results suggest that E. campestre extracts act as non-specific donators for hydrogen atoms or electrons in the DPPH-assay. When compared to the reference substances, the Eryngium extracts were found to be less efficient. Nevertheless, the existing scavenging effects of the

### Table 6. The content of minerals in Eryngii herba and Eryngii radix after mineralization in micro-wave digestion system (MW) (w/mg/kg)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cd</th>
<th>Co</th>
<th>Cr</th>
<th>Cu</th>
<th>Fe</th>
<th>K</th>
<th>Mn</th>
<th>Na</th>
<th>Pb</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eryngii herba</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EH1/02</td>
<td>0.11</td>
<td>0.08</td>
<td>0.70</td>
<td>8.17</td>
<td>69.9</td>
<td>1963</td>
<td>33.78</td>
<td>43.66</td>
<td>0.17</td>
<td>17.19</td>
</tr>
<tr>
<td>EH2/02</td>
<td>0.14</td>
<td>0.10</td>
<td>1.57</td>
<td>9.90</td>
<td>119.7</td>
<td>2167</td>
<td>47.68</td>
<td>81.50</td>
<td>0.75</td>
<td>19.17</td>
</tr>
<tr>
<td>EH3/02</td>
<td>0.11</td>
<td>0.08</td>
<td>1.58</td>
<td>8.26</td>
<td>114.5</td>
<td>2228</td>
<td>44.67</td>
<td>56.74</td>
<td>0.28</td>
<td>20.40</td>
</tr>
<tr>
<td>EH1/03</td>
<td>0.067</td>
<td>0.09</td>
<td>1.55</td>
<td>11.17</td>
<td>148.6</td>
<td>1774</td>
<td>32.51</td>
<td>46.15</td>
<td>0.18</td>
<td>15.99</td>
</tr>
<tr>
<td>EH2/03</td>
<td>0.06</td>
<td>0.13</td>
<td>1.36</td>
<td>10.39</td>
<td>196.8</td>
<td>1765</td>
<td>31.46</td>
<td>62.87</td>
<td>0.97</td>
<td>15.93</td>
</tr>
<tr>
<td>EH3/03</td>
<td>0.10</td>
<td>0.10</td>
<td>1.23</td>
<td>10.74</td>
<td>162.5</td>
<td>1797</td>
<td>32.86</td>
<td>94.70</td>
<td>1.33</td>
<td>17.47</td>
</tr>
<tr>
<td>Eryngii radix</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER1/02</td>
<td>0.16</td>
<td>0.50</td>
<td>3.19</td>
<td>12.45</td>
<td>213.7</td>
<td>934</td>
<td>26.11</td>
<td>590.9</td>
<td>0.14</td>
<td>25.29</td>
</tr>
<tr>
<td>ER2/02</td>
<td>0.12</td>
<td>0.44</td>
<td>4.33</td>
<td>11.29</td>
<td>209.7</td>
<td>849</td>
<td>25.19</td>
<td>551.85</td>
<td>0.36</td>
<td>22.63</td>
</tr>
<tr>
<td>ER3/02</td>
<td>0.10</td>
<td>0.49</td>
<td>3.82</td>
<td>10.64</td>
<td>325.7</td>
<td>744</td>
<td>26.02</td>
<td>492.99</td>
<td>0.10</td>
<td>20.95</td>
</tr>
<tr>
<td>ER1/03</td>
<td>0.04</td>
<td>0.24</td>
<td>2.53</td>
<td>9.16</td>
<td>198.9</td>
<td>932</td>
<td>41.41</td>
<td>170.70</td>
<td>0.46</td>
<td>16.57</td>
</tr>
<tr>
<td>ER2/03</td>
<td>0.09</td>
<td>0.18</td>
<td>2.45</td>
<td>9.52</td>
<td>208.7</td>
<td>962</td>
<td>41.18</td>
<td>172.38</td>
<td>1.76</td>
<td>16.68</td>
</tr>
<tr>
<td>ER3/03</td>
<td>0.06</td>
<td>0.19</td>
<td>2.03</td>
<td>10.43</td>
<td>183.3</td>
<td>910</td>
<td>41.19</td>
<td>218.67</td>
<td>0.62</td>
<td>15.56</td>
</tr>
</tbody>
</table>

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examined extracts probably could be attributed to the presence of flavonoids, but also could be resulted of the activity of other secondary biomolecules present in the extracts.

Compared to literature data, different Eryngium species manifest different radical scavenging activity. Thus, the methanol extracts of leaves and inflorescence of Eryngium caucasicum at flowering stage show remarkable activity with IC$_{50}$ = 0.15±0.01 mg ml$^{-1}$ for leaves and 0.39±0.02 mg ml$^{-1}$ for inflorescence (Ebrahimzadeh et al. 2009; Nabavi et al. 2009). Radical scavenging activity of E. maritimum methanol extract was investigated, as well, revealing IC$_{50}$ = 0.28 mg ml$^{-1}$ in the ABTS assay (Meot-Duros et al. 2008). The effect of Eryngium extracts on the inhibition of hydroxyl radical production (OH$^-$) was assessed by the iron (II)–dependent deoxyribose damage assay. It is well known that the Fenton reaction generates hydroxyl radicals (OH$^-$), which degrade deoxyribose, using Fe$^{2+}$ salts as an important catalytic component (Halliwell and Gutteridge, 1981). Oxygen radicals may attack sugar, which leads to sugar fragmentation. Addition of transition metal ions such as iron at low concentrations to deoxyribose, causes degradation of sugar into malondialdehyde and other related compounds which form a chromogen with thiobarbituric acid (TBA). Table 7 presents the results of the effects of examined Eryngium extracts as well as that of reference substances on OH$^-$ radical production. They show that both extracts of Eryngium campestre and reference substances inhibited the production of OH$^-$ radicals. The strongest inhibitory activity was exhibited by BHA (52%). Both extracts of Eryngium campestre exhibited significant inhibitory effect, 45% and 50%, respectively, higher than the percentage of inhibition obtained by quercetin (42%). Previously, it has been shown that quercetin and its glycosides exert inhibitory activity against lipid peroxidation (Cook and Samman, 1996; Dangles, 2000).

### Table 7. Free radical scavenging activity of the extracts of Eryngium campestre in DPPH assay and inhibition of OH$^-$ radical production (%)

<table>
<thead>
<tr>
<th>Sample</th>
<th>DPPH assay (IC$_{50}$ mg/ml)</th>
<th>OH$^-$ (% of inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eryngii radix</td>
<td>0.72</td>
<td>45.00</td>
</tr>
<tr>
<td>Eryngii herba</td>
<td>1.14</td>
<td>50.11</td>
</tr>
<tr>
<td>Quercetin</td>
<td>0.06</td>
<td>42.05</td>
</tr>
<tr>
<td>BHA</td>
<td>0.15</td>
<td>52.09</td>
</tr>
</tbody>
</table>

### Table 8. Effect of ethanol extracts of Eryngium campestre in comparison to BHA and quercetin on the oxidation of β-carotene-bleaching assay

<table>
<thead>
<tr>
<th>Sample</th>
<th>A$_{470}$ (% of initial value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time (min)</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Eryngii radix</td>
<td>100</td>
</tr>
<tr>
<td>Eryngii herba</td>
<td>100</td>
</tr>
<tr>
<td>Quercetin</td>
<td>100</td>
</tr>
<tr>
<td>BHA</td>
<td>100</td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
</tr>
</tbody>
</table>

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Inhibition of lipid peroxidation (LP)

Antioxidant activity of ethanol extracts of the aerial part and root of E. campestre was examined over the inhibition of spontaneous lipid peroxidation in rat liver homogenate as well, measuring the content of formed MDA by TBA assay (TBARS). The results given in Table 9 showed that the content of formed MDA was lower when the extract of root or aerial part were added into the system (3.67 ± 0.04 and 4.05±0.07 nmol ml⁻¹, respectively), but still the levels of MDA were significantly higher than those obtained when quercetin was used as an antioxidant (3.12 ± 0.04 nmol ml⁻¹).

Table 9. The content of MDA (nmol mg⁻¹) and the inhibition of lipid peroxidation (%) in rat liver homogenate by ethanol extracts of aerial part and root of E. campestre

<table>
<thead>
<tr>
<th>Sample</th>
<th>TBARS – homogenate (nmol MDA mg protein⁻¹)</th>
<th>Inhibition of LP (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (ethanol)</td>
<td>4.60 ± 0.02</td>
<td>0.0</td>
</tr>
<tr>
<td>Eryngii radix</td>
<td>3.67 ± 0.04</td>
<td>20.0</td>
</tr>
<tr>
<td>Eryngii herba</td>
<td>4.05 ± 0.07</td>
<td>12.2</td>
</tr>
<tr>
<td>Quercetin</td>
<td>3.12 ± 0.04</td>
<td>36.9</td>
</tr>
</tbody>
</table>

The range of inhibition of lipid peroxidation in the rat liver homogenate was 20% and 12% for the extract of root and aerial part of E. campestre, respectively. Obtained values were lower than that of quercetin (36.9%). Compared to the recently published data on the activity of some wild plants such as Calamintha nepeta, Calamintha gradi-flora and Micromeria cristata, higher inhibition activity of ethanol extracts on lipid peroxidation in the same biological system was exhibited (20.35%, 25.6% and 29.6% of inhibition, respectively) (Kadifkova Panovska 2004). Besides evident free radical scavenging activity against DPPH and hydroxyl (OH⁻) radical, antioxidant activity of Eryngium campestre in both non-biological (β-carotene/linoleic acid) and biological (rat liver homogenate) system manifested a low capacity.

Conclusion

Analysis of the chemical composition of the aerial part and root of Eryngium campestre from Kosovo showed that the content of total ash, ash insoluble in hydrochloric acid, loss on drying and the content of water extract were relatively constant regards the differences in the characteristics of the locations and year of collection. Samples of Eryngii herba gave lower percentage of water extract (18.3-24.5%) in comparison to the samples of Eryngii radix (29.6-36.6%). The analysis of mineral content (Zn, Fe, Cu, Mn, Ni, K, Co, Pb and Cr) showed that different part of E. campestre accumulate different amount of investigated minerals. Evaluation of radical scavenging and antioxidant activity showed that higher radical-scavenging activity against DPPH radical has been presented by the ethanol extract of root of E. campestre (IC₅₀ = 0.72 mg ml⁻¹) compared to the aerial part of the plant (IC₅₀ = 1.14 mg ml⁻¹). Furthermore, the inhibition capacity on the production of hydroxyl radical in deoxyribose system was found to be strong (50% and 45% for aerial part and root ethanol extract, respectively). However, both ethanol extracts of E. campestre from aerial part and root, exhibited low antioxidant activity in β-carotene/linoleic acid system as well as low capacity for inhibition of spontaneous lipid peroxidation in rat liver homogenate.

Acknowledgment

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References

F. Nebija, G. Stefkov, M. Karapandzova, T. Stafilov, T. Kadifkova Panovska, S. Kulevanova


Chemical characterization and antioxidant activity of *Eryngium campestre* L., Apiaceae from Kosovo

1(6), 435-439.


Suciu, S., Bodoki, E., Vlase, L. 2006. Comparative phytochemical study on *Eryngium* sp. from Romania, 4th Conference of Medicinal and Aromatic Plants of South-East European Countries, Book of abstracts, 64-65.


Макед. фарм. билт., 55 (1, 2) 22 - 32 (2009)
Резиме

Хемиска карактеризација и антиоксидативна активност на Eryngium campestre L., Apiaceae од Косово

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3Институт за хемија, ПМФ УКИМ, Скопје, Република Македонија

Ключни зборови: Eryngium campestre, флавоноиди, минерали, DPPH тест, антиоксидативна активност, β-каротен-избелувачки тест, TBA тест.

Оваа студија се однесува на дефинирање на хемиски состав и утврдување на антиоксидативна активност in vitro на екстракти подготвени од надземниот дел и од коренот на Eryngium campestre L. (Apiaceae) од Косово. Анализата на хемискиот состав вклучува определување на вкупен пепел, пепел нерастворлив во хлороводородна киселина, губиток со сушење и содржина на вoden екстракт, како и определување на содржина на флавоноиди во надземниот дел. Составот на минералите (Zn, Fe, Cu, Mn, Ni, K, Co, Pb, Cd и Cr) во надземниот дел и во коренот се определени со користење на атомска апсорпциона спектроскопија (AAS и ETAAS). Утврдено е дека различните делови од E. campestre акумулираат различни количества од испитуваните минерали. Антиоксидативната активност е испитувана со четири методи: DPPH тест, инхибиција на продукција на хидроксил радикал, β-каротен-избелувачки тест и инхибиција на липидна пероксидација (TBA тест). Во DPPH системот етанолните екстракти од коренот покажуваат подобра радикал-фаќачка активност (IC50 = 0.72 mg ml1) во споредба со соодветните екстракти од надземниот дел (IC50 = 1.14 mg ml1). Од друга страна, етанолниот екстракт од надземниот дел покажува поголем инхибирачки капацитет врз продукцијата на хидроксил радикалот во системот од дезоксирибоза во споредба со екстрактот од коренот (50% и 45%, соодветно). Двата етанолни екстракти од E. campestre покажуваат ниска антиоксидативна активност во β-каротен-избелувачки тест, како и низок капацитет за инхибирање на спонтана липидна пероксидација во хомогенат од црн дроб од стаорец.
Seasonal variation of flavonoids in *Teucrium polium* L. (Lamiaceae)

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Abstract

The aim of the present study was identification of flavone aglycones and determination of the content of each and the content of total flavonoids as well as investigation of the eventual seasonal variations of flavonoids in *Teucrium polium* L. (Lamiaceae). The plant samples were collected at six different locations in Republic of Macedonia, during summer in 1999, 2000 and 2003. For determination of seasonal variations, the samples were collected in v. Koleshino, in 2004, each month during the whole season. Six flavone aglycones (luteolin, apigenin, diosmetin, cirsiliol, cirsimaritin and cirsilineol) were identified in the hydrolyzed extracts of the over ground part of *Teucrium polium* by HPLC method. The most abundant flavone was luteolin, followed by apigenin and cirsimaritin. Great seasonal variations were found in the content of each and in the content of total amount of flavonoids. The most abundant flavone during the whole season was luteolin with the highest content in May. The content of total flavonoids was the highest in the period from May to July, which could be recommended as the most convenience period in the season for collecting of the plant material from *Teucrium polium*.

Key words: *Teucrium polium*, flavonoids, HPLC analysis, seasonal variation

Introduction

*Teucrium polium* L. (Lamiaceae) is a sub-shrub plant native to the Mediterranean region and the Middle East. In Republic of Macedonia it is widely distributed and traditionally used by native inhabitants, as herbal hypoglycemic tea. The decoctum of *T. polium* is used as an appetite especially in children and also as a spice. Some biological and therapeutic effects have been reported for the plant such as antioxidant (Esmaeili et al., 2009; Ardestani et al., 2008), antiinflammatory (Tariq et al., 1989; Capasso et al., 1983), antinociceptive (Baluchnejadmajarad et al., 2005; Abdollahi et al., 2003), antipyretic (Aggelis et al., 1998; Autore et al., 1984), anti-microbial (Autore et al., 1984), hypolipidemic (Rasekh et al., 2001), hepatoprotective (Panovska et al., 2007), cytotoxic and apoptotic effects (Rajabalian et al., 2008). The plant poses complex chemical composition with presence of new clerodane type diterpenes (Malakov and Papanov, 1983; Marquez and Valverde, 1979), essential oil with dominating sesquiterpene alcohols and pinenes (Cozzani et al., 2005; Moghtader, 2009; Kabouche et al., 2007), phenylethanoid glycosides such as verbascoside and poliumoside (Oganesyan et al., 1991), flavone glycosides with highly methylated aglycons (Verykokidou-Vitsaropoulou and Vajias, 1986; Rizk et al., 1986; Kawashly et al., 1999; Harborne et al., 1986; Shariffar et al., 2009), etc.

Flavonoids are representing the most important group of active components of *Teucrium* species, and many of the activities of these plants are attributed to the flavonoid
class of secondary metabolites. It is also well known that the composition and the content of the flavonoids in plant material could be variable depending on the season, location and environmental condition of plant growth as well as the influence of other different factors (Liu et al., 1994; De Castro, et al., 2006; Bagdonaite et al., 2009). Seasonal variations of flavonoids were also studied in different plant species (Luengas-Caicedo et al., 2007; Akabori, 1978; Ioku et al., 2005; Xu et al., 2009).

Until now, there is no published data about the seasonal variation in the composition and the content of the flavonoids in *Teucrium polium*. Taking into account all of these, the aim of the present study were identification, quantification and determination of the seasonal variations of the flavonoids in *Teucrium polium* from Macedonian origin.

**Material and methods**

**Plant material**

The over-ground parts of the plant of 6 different populations of *T. polium* were collected during the summer of 1999, 2000, 2003 and 2004 (Table 1). The plant material was air dried, packed in paper bags and kept in a dark and cool place until analysis. Plant identity was verified and voucher specimens were deposited at the Institute of Pharmacognosy, Faculty of Pharmacy, Skopje, R. Macedonia.

**Reagents and authentic samples**

Reagents of HPLC purity were purchased from Sigma Chemical Co. (Germany). Authentic substances apigenin, luteolin, chryseriol, diosmetin, acacetin, genkwa nin, naringenin and eryiodictiol were the products of Extrasynthese (France). Cirsimaritin, cirsilineol, and 5,4’-OH and 6,7,8,3’-OCH₃ flavones were kindly donated by Dr. B. Voisin from the Laboratoire de Phytochimie, U. E. R. des Sciences de la Nature, Université Claude Bernard Lyon, France.

**Preparation of hydrolyzed extracts**

Grounded plant material (1 g) was extracted in an Erlemeyer flask with reflux in a water bath with mixture of 25 ml acetone, 1 ml of concentrated HCl and 0.5 ml of 1% solution of urotropine. The extraction was performed twice, first for 40 min at 60 °C and then for 20 minutes more on the same temperature. The extracts were cooled, filtered and transferred to a 50 ml volumetric flask and filled up with acetone. 10 ml were transferred to a separating funnel. Water (25 ml) was added and extraction with ethyl acetate was repeated 3 times with 10 ml portion. The ethyl acetate fractions were collected, washed three times with 25 ml of water, then dried with anhydrous Na₂SO₄, filtered, and evaporated to dryness under low pressure. The residue was dissolved in 1 ml methanol and the solution was used for analyses of flavonoid aglycones by HPLC.

**HPLC analysis**

Flavonoid aglycones in the hydrolyzed extracts were analyzed by the HPLC method, using a Varian HPLC system equipped with a ternary pump Model 9012 and UV diode-array detector Model 9065. A reverse phase column C18 (250 x 4.6 mm, 5 µm particles) was used. The column was stabilized in thermostat at 35 ºC with heater of column (CH-30) and temperature controller (TC-45). The mobile phase consisted of H₂O with pH adjusted to 3 with H₃PO₄ (A) and CH₃CN (B). The elution program for extracts screening was the following: 0–5 min 70% A; 10–20 min 65% A; 25–30 min 55% A; 40–48 min 35% A. The flow rate was 1 ml min⁻¹, the temperature was set to 35 ºC and the injection volume was 20 µl.

The elution was monitored in the whole UV range and the chromatograms for flavone screening were best seen at 348 nm, which is in the region where flavones exhibit an absorption maximum. Identification was made according to the retention times and UV spectra of the components compared to those of authentic samples of flavonoids. Semi-quantification of flavones was performed on the basis of the peak areas of flavones in the HPLC chromatograms at 348 nm.

**Table 1. The locations of collection of samples of *T. polium***

<table>
<thead>
<tr>
<th>Species</th>
<th>Voucher specimen</th>
<th>Location</th>
<th>Mount and year of collection</th>
<th>Collection for determination of seasonal variation</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Teucrium polium</em></td>
<td>T₄</td>
<td>v. Janche</td>
<td>July - 1999</td>
<td></td>
</tr>
<tr>
<td><em>Teucrium polium</em></td>
<td>T₉</td>
<td>v. Rashtak</td>
<td>July - 2000</td>
<td></td>
</tr>
<tr>
<td><em>Teucrium polium</em></td>
<td>T₁₁</td>
<td>v. Gari</td>
<td>July - 2000</td>
<td></td>
</tr>
<tr>
<td><em>Teucrium polium</em></td>
<td>T₁₂</td>
<td>Alshar</td>
<td>July - 2000</td>
<td></td>
</tr>
<tr>
<td><em>Teucrium polium</em></td>
<td>T₁₅</td>
<td>Arkutino</td>
<td>July - 2000</td>
<td></td>
</tr>
</tbody>
</table>
Results and discussion

HPLC analysis of flavonoids

The identification of flavone aglycons in the extracts of *T. polium*, was done by comparing the retention times and UV spectral data of the extract components with those of authentic flavonoid substances. Two mixtures of authentic substances of flavonoids were used, labeled as St₁ and St₂. The composition of the mixtures and relating retention times of flavonoids are presented in Table 2. The HPLC chromatograms of both mixtures of standards are presented on Fig.1.

Six flavone aglycons, luteolin, apigenin, cirsiliol, diosmetin, cirsimaritin and cirsilineol (Table 3) were identified in the hydrolyzed extracts of *T. polium*. As the authentic samples for the component cirsiliol was not available, identification was made using previously published data by Stefova et al. (2007) and other literature data (Verykokidou-Vitsaropoulou and Vajias, 1986; Rizk et al., 1986).

### Table 2. Retention times (tᵣ) of the components in two mixture of authentic samples of flavonoids (St₁ and St₂)

<table>
<thead>
<tr>
<th>Mixture</th>
<th>Structure</th>
<th>tᵣ/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>St₁</td>
<td>Apigenin 5,7,4’-OH flavone</td>
<td>15,59</td>
</tr>
<tr>
<td></td>
<td>Diosmetin 5,7,3’-OH, 4’-OCH₃ flavone</td>
<td>17,65</td>
</tr>
<tr>
<td></td>
<td>Cirsimaritin 5,4’-OH 6,7-OCH₃- flavone</td>
<td>26,77</td>
</tr>
<tr>
<td></td>
<td>Cirsilineol 5,4’-OH 6,7,3’-OCH₃- flavone</td>
<td>28,43</td>
</tr>
<tr>
<td></td>
<td>Genkwanin 5, 4’-OH 7-OCH₃ flavone</td>
<td>32,98</td>
</tr>
<tr>
<td>St₂</td>
<td>Eryodictiol 5,7,3’,4’-OH flavanone</td>
<td>9,15</td>
</tr>
<tr>
<td></td>
<td>Luteolin 5,7,3’,4’-OH flavone</td>
<td>10,79</td>
</tr>
<tr>
<td></td>
<td>Naringenin 5,7,4’-OH flavanone</td>
<td>13,82</td>
</tr>
<tr>
<td></td>
<td>Apigenin 5,7,4’-OH flavone</td>
<td>15,73</td>
</tr>
<tr>
<td></td>
<td>Chryseriol 5,7,4’-OH, 3’-OCH₃ flavone</td>
<td>17,31</td>
</tr>
<tr>
<td></td>
<td>Diosmetin 5,7,3’-OH, 4’-OCH₃ flavone</td>
<td>17,77</td>
</tr>
<tr>
<td></td>
<td>Acacetin 5,7-OH 4’-OCH₃, flavone</td>
<td>32,09</td>
</tr>
<tr>
<td></td>
<td>Genkwanin 5, 4’-OH 7-OCH₃, flavone</td>
<td>32,98</td>
</tr>
</tbody>
</table>

![Fig. 1. HPLC chromatograms (348 nm) of two mixtures of standard substances of flavones. St₁: 1-apigenin, 2 – diosmetin, 3- cirsimaritin, 4 – cirsilineol, 5 – genkwanin; St₂: 1 – eryodictiol, 2 – luteolin, 3 – naringenin, 4 – apigenin, 5 – chryseriol, 6 – diosmetin, 7 – acacetin, 8 - genkwanin](image)
The HPLC chromatogram of hydrolyzed extract of *T. polium* is presented at Fig. 2. The results of semi-quantitative analysis of the content of each flavones and the amount of total flavonoids are presented in Table 3.

Comparing to literature data, flavones identified in extracts of Macedonian *T. polium* are well known components of this species. Previously, in *T. polium* cirsimaritin, cirsiliol, cirsilineol, 5-hydroxy-6,7,3',4'-tetramethoxyflavone, salvigenin, apigenin 5-galloyl-glycoside, apigenin-7-glycoside, vicenin-2 and luteolin-7-glycoside were reported (Esmaeili and Yazdanparast, 2004; Esmaeili at al., 2009; Harborne et al., 1986; Verykokidou-Vitsaropoulou and Vajias, 1986; Rizk et al., 1986, Kwahtsy et al., 1997; Panovska et al., 2007).

**Seasonal variation of flavonoids**

For determination of the eventually presented seasonal variation, the composition and the content of flavonoids were determined by HPLC in the samples of *T. polium* collected from the same location (v. Koleshino, south-eastern part of Republic of Macedonia), each month (except June) during 2004. The same HPLC method mentioned above was used and the hydrolyzed extracts were prepared on a same way, as it was made in a purpose of identification and quantification of the flavonoids.

In all investigated samples of *T. polium* that were collected for purpose of determination of seasonal variations, the same flavone aglycons were identified (Table 4), but in variable ratio during the season. For expression of the relative ratio of flavones, the peak area of cirsilineol measured in October was the lowest, and this area was expressed as one. The all other peak areas of all determined flavones were expressed in appropriately larger values presented in Table 4. The content of total flavonoids is presented as a sum of these numerical values for each month separately. The seasonal variation in the content of six flavones aglycones in *T. polium* is presented at Fig. 4, while the relative abundance of the flavonoids is presented on Fig. 5.

**Table 3.** Semi-quantitative determination of flavones aglycons in hydrolyzed extracts of *T. polium*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Luteolin</th>
<th>Apigenin</th>
<th>Cirsiliol</th>
<th>Diosmetin</th>
<th>Cirsimaritin</th>
<th>Cirsilineol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tp</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>tr</td>
</tr>
<tr>
<td>T₃</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>tr</td>
<td>+</td>
<td>tr</td>
</tr>
<tr>
<td>T₉</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>tr</td>
</tr>
<tr>
<td>T₁₁</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>tr</td>
</tr>
<tr>
<td>T₁₂</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>tr</td>
<td>+</td>
<td>tr</td>
</tr>
<tr>
<td>T₁₅</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>tr</td>
<td>+</td>
<td>tr</td>
</tr>
</tbody>
</table>

(++) - Dominantly present; (+) - present; tr – presented in traces; (-) – not detected

**Fig. 2.** HPLC chromatogram of hydrolyzed extract of *T. polium* – C, and mixtures of standard substances: St₁ – B and St₂ – A.
Table 4. Total amount and relative abundance of flavone aglycones during the one season of *Teucrium polium*, with normalized peak areas values of flavonoids accounted on peak area of cirsimaritin, expressed as value one.

<table>
<thead>
<tr>
<th>Mounts</th>
<th>Luteolin</th>
<th>Apigenin</th>
<th>Cirsiliol</th>
<th>Diosmetin</th>
<th>Cirsimaritin</th>
<th>Cirsilineol</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>64</td>
<td>2</td>
<td>17</td>
<td>5</td>
<td>12</td>
<td>5</td>
<td>105</td>
</tr>
<tr>
<td>IV</td>
<td>95</td>
<td>5</td>
<td>38</td>
<td>19</td>
<td>23</td>
<td>9</td>
<td>198</td>
</tr>
<tr>
<td>V</td>
<td>107</td>
<td>30</td>
<td>38</td>
<td>17</td>
<td>28</td>
<td>6</td>
<td>226</td>
</tr>
<tr>
<td>VI</td>
<td>90</td>
<td>56</td>
<td>22</td>
<td>8</td>
<td>42</td>
<td>17</td>
<td>242</td>
</tr>
<tr>
<td>VII</td>
<td>60</td>
<td>21</td>
<td>11</td>
<td>5</td>
<td>17</td>
<td>7</td>
<td>143</td>
</tr>
<tr>
<td>VIII</td>
<td>39</td>
<td>25</td>
<td>6</td>
<td>3</td>
<td>18</td>
<td>3</td>
<td>99</td>
</tr>
<tr>
<td>IX</td>
<td>28</td>
<td>14</td>
<td>22</td>
<td>4</td>
<td>5</td>
<td>1</td>
<td>58</td>
</tr>
<tr>
<td>X</td>
<td>41</td>
<td>28</td>
<td>11</td>
<td>6</td>
<td>21</td>
<td>1</td>
<td>129</td>
</tr>
<tr>
<td>XI</td>
<td>102</td>
<td>6</td>
<td>43</td>
<td>6</td>
<td>27</td>
<td>9</td>
<td>200</td>
</tr>
<tr>
<td>XII</td>
<td>82</td>
<td>4</td>
<td>25</td>
<td>13</td>
<td>21</td>
<td>8</td>
<td>155</td>
</tr>
<tr>
<td>I</td>
<td>59</td>
<td>3</td>
<td>19</td>
<td>7</td>
<td>15</td>
<td>8</td>
<td>111</td>
</tr>
</tbody>
</table>

Fig. 3. Seasonal variations in the content of six flavone aglycones in *Teucrium polium* (1-12 number of months)

Fig. 4. Relative ratio of the flavonoids in *Teucrium polium* during one season (1-12 numbers of months)
From the presented results (Table 4 and Fig. 3 and Fig. 4), it could be noticed that there are no qualitative variations in the flavone aglycons in T. polium during one season, as all six flavone aglycons were identified in each month. On the other side, the content of the each flavone aglycone and the content of the total flavonoids vary a lot during the whole season. For instance, cirsilineol ranged from 1 in October to 9 in April and in December, cirsimaritin from 5 in October to 42 in July, while luteolin from 28 in October rise to 107 in May. The variations of each flavonoid were different and the highest amount of luteolin was found in May, for apigenin in July, for cirsiliol and diosmetin in April, for cirsimaritin in July and for cirsimilol in November. The content of total flavonoids was the highest in the period from May to July, when in the composition of flavonoids, luteolin was dominated aglycone, followed by apigenin and than by cirsiliol and cirsimaritin. From the Fig. 5, it could be seen that luteolin was the dominated aglycon during the whole season. According to these results, the over ground parts of T. polium collected in the period from May to July will have the highest quantity of total flavonoids. This period correspond to flowering phase of T. polium. The period from August to November is period of fruiting and it is not convenience for collecting of the plant material, as the content of total flavonoids declines.

Conclusion

Six flavone aglycones (luteolin, apigenin, diosmetin, cirsiliol, cirsimaritin and cirsimarine) were identified by HPLC method in the hydrolyzed extracts of the over ground part of Teucrium polium from R. Macedonia. The most abundant flavone was luteolin, followed by apigenin and cirsimaritin. Great seasonal variations were found in the content of each flavonoid and in the total flavonoids amount. The content of all flavonoids was the highest in the period from May to July, which could be recommended as the most convenient period for collecting of plant material from T. polium, rich in flavonoids. Opposite to this, the period of maturation was characterized with the lowest total amount of the flavonoids and represents the period in the year when the collection of plant material should be highly avoided.

References


Maced. pharm. bull., 55 (1, 2) 33 - 40 (2009)
Seasonal variation of flavonoids in *Teucrium polium* L. (Lamiaceae)


Rajabalian, S., 2008. Methanolic extract of *Teucrium polium* L. potentiates the cytotoxic and apoptotic effects of anticancer drugs of vincristine, vinblastine and doxorubicin against a panel of cancerous cell lines. Exp. Oncol. 30, 133-138.


Резиме

Сезонски варијации на flavоноиди во *Teucrium polium* L. (Lamiaceae)

Главни членови: *Teucrium polium*, flavоноиди, HPLC анализа, сезонски варијации

Целта на трудот е идентифицирање на flavоноидни агликони и определување на содржина на поединечни и на вкупни flavоноиди, како и испитување на можни сезонски варијации на flavоноиди во *Teucrium polium* L. (Lamiaceae). Примероците од растението се собирани од шест различни локалитети во Република Македонија, во текот на летото во 1999, во 2000 и во 2003 година. За утврдување на сезонските варијации примероците се собирани во с. Колешино, во 2004 година, секој месец во текот на целата година. Во хидролизирани отстапки од надземниот дел на *Teucrium polium*, со HPLC метод се идентификуваат шест flavоноидни агликони (лутеолин, апигенин, диосметин, цирсилиол, цирсимаритин и цирсилинеол), меѓу кои доминира лутеолин, а потоа апигенин и цирсимаритин. Во содржината на поединечните, како и во содржината на вкупниот flavоноиди најдени се големи сезонски варијации. Доминантен flavоноид во текот на целата сезона е лутеолин, најмногу застапен во текот на месец мај. Содржината на вкупните flavоноиди е најголема во периодот од мај до јули и овој период може да се препорача како најсоодветен период во вегетациониот развој на растението за собирање на растителниот материјал од *Teucrium polium*.
Фармакогностички интересни ендемични растенија во Република Македонија

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Анстракт

Во флората во Република Македонија има околу 3 200 видови во 147 фамилии. Од нив според едни извори, 115 се позната ендемични виши растенија од кои 114 припаѓаат во групата скриеносемени. Според други извори има 135 видови ендемични растенија од кои се смета дека околу 111 се локални ендемични видови, а 24 во пограничните планини. Точниот број се разликува со низините посебно се исклучува околината на Прилеп.

И покрај богатството на ендемични и реликтни видови ендемични видови не се објавени било какви фармакогностички податоци за овие растенија. Од наведените ендемични видови, околу 30 би можеле да бидат фармакогностички интересни за испитување на хемискиот состав, изолација на потенцијално активни супстанции и испитување на биолошко-фармаколошката активност. Новите инструменти и техники што денес се користат во фармакогностички интересни видови овозможуваат да се користат низините посебно се истакнува околината на Прилеп.

Дополнителен предизвик претставува изготвување на соодветна програма за заштита на сите ендемични растенија, со посебен акцент на фармакогностички интересните видови.

Ключни зборови: ендемични растителни видови, Република Македонија, фармакогнозија

Вовед

Ендемизм на растенија е еколошка состојба на уникатност на ботаничките видови врзани за определено географско подрачје, национални граници или географски зони, изолирани региони и слично, при што истите не можат да се најдат на ниво друго подрачје, регион или област. Спротиво на ендемични растенија кои се врзани за ограничени географски подрачја и специфички геолошки средини, космополитските растенија се широко присутни во речиси сите географски и климатски зони. Физичките, биолошките и климатските фактори можат да променат во голема мера за појава на ендемизмот. Ендемичните видови можат посебно да се развијат на биолошки изолирани места како што се на пример, ендемските растенија на островите поради нивната географска положба (Endemism, 2009; Endemic Plants 2003; Definition of endemic in US English dictionary, 2009; Endemic, eLook Online Dictionary). Покрај ендемични, разликуваме уште една специфична група растенија означени како реликтни. Реликтни растенија се специфична група растенија што преживееле драматични промени во средината во кои живеат и се суштите уште се држат на местото на кое биле распределени на нивните видови и се развијат на биолошки изолирани места. Ендемичните видови се специфични за определено географско подрачје и имаат уникатност на ботаничките видови.
Освен што се интереси како флористички елементи, од таксономски аспект и како видови што го збогатуваат флорирот биодиверзитет, некои ендемични и релктни растенија се интереси и од фармакобиологички аспект. Можност да се пручува хемизмот и биохемика на активност на ваквите видови овозможува добивање корисни научни податоци за збогатување на научниот фонд за испитувањата видови од флората на Република Македонија, но претставува и можност да се добијат информации кои можат да се користат во хемотаксономски цели, да се изнајдат нови соединенија и проценети потенцијал на нивната биолошка активност, што претставува уникатен научен податок со капацитет од пошироко значење во медицински или во други цели. Со оглед на можностите што ги нудат новите инструментални, високо софистицирани техники, потребното количество материјал за хемиски анализ е многу мало (помало од еден грам) што не претставува дополнителна закана за нанесување штета на овие растенија кога материјалот за испитување се собира од природните наоѓалишта. Оттука, цел на овој труд е потенцирање на ендемичните и релктни растенија од Република Македонија, а одделно внимание во целина се однесува подеднакво и на микроорганизмите, нижите растенија како што се лишаите, габите и вишите растенија. Тоа се однесува подеднакво и на микроорганизмите, нижите растенија како што се лишаите, габите и вишите растенија.

Краток преглед на богатството на биолошка разновидност во Република Македонија

Богатството од екосистеми, живеалишта, заедници и посебни растителни места во Република Македонија превзема разнообразни роли. Во суб-медитеранскиот појас кој се допаѓа во градот Солун, на нивната биолошка разновидност се акцентираат четири области: топла континентална област, подевената планинска област, високата планинска област и планинската област над 1 650 метри надморска висина. Во континенталниот појас се разликуваат четири области: планинската област над 1 650 метри надморска висина, високата планинска област над 1 650 метри надморска висина, континенталната област над 2 250 метри надморска висина и планинската област над 2 250 метри надморска висина. Во алпскиот појас се разликуваат две области: планинската област над 2 250 метри надморска висина и планинската област над 3 000 метри надморска висина. Во суб-медитеранскиот појас се разликуваат четири области: планинската област над 1 650 метри надморска висина, високата планинска област над 1 650 метри надморска висина и планинската област над 2 250 метри надморска висина.

По својата таксономска припадност, растителните зони на Република Македонија се поделени на четири основни групи: макрови, папрати, лишаи и архей. Табелата ги прикажува кое е растениеот од флората на Република Македонија.
и 13 таксони - 7 видови и 6 пониски таксони), папрати (Filibiniae, со 15 фамилии, 21 род и 60 таксони - 42 видови и 18 пониски таксони), Coniferophyta – со 4 фамилии, 6 родови и 22 таксони - 15 видови и 7 пониски таксони, 50 фамилии од класата Dicotyledoneae со 235 родови и 1 630 таксони – 1 028 видови и 602 пониски таксони. Во рамките на Македонската Академија на науките и уметностите преку издането „Флора на Република Македонија“ претставени се резултатите од интензивните истражувања и во останатите фамилии во оваа класа (Мицевски, 1985; 1993). Ендемичните и релектните видови се изучувани чрез значајни компоненти во растителната разновидност на Македонија. Според податоците на Матевски (1990) и Матевски и Костадиновски (1996) има 135 ендемични виш растителни видови на територијата на Република Македонија од кои 111 се наоѓаат на територијата на Македонија, а останатите видови се наоѓаат на плаантинските места на границите со Грција, Албанија, Србија, Косово и Бугарија.

Ендемични, релектни и ретки растителни видови во Р. Македонија


Табела 2. Позначајни релектни растителни видови во Р. Македонија

<table>
<thead>
<tr>
<th>Релектни растителни видови</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymus oehmius*</td>
</tr>
<tr>
<td>Crocus cvijič*</td>
</tr>
<tr>
<td>Pinus heldreichii*</td>
</tr>
<tr>
<td>Ruscus hypoglossum*</td>
</tr>
<tr>
<td>Gentiana asclepiadea*</td>
</tr>
<tr>
<td>Viola kosanini*</td>
</tr>
<tr>
<td>Acer heldreichii*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Бореал, Арктички релектни растителни видови</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trifolium pilezii*</td>
</tr>
<tr>
<td>Dryas octopetala</td>
</tr>
<tr>
<td>Arctostaphylos uva ursi*</td>
</tr>
<tr>
<td>Salix reticulata*</td>
</tr>
<tr>
<td>Carex laevis</td>
</tr>
<tr>
<td>Arabis alpina*</td>
</tr>
</tbody>
</table>

**видови коишто имаат своите сродници во истото род што се фармакогностички значајни**

Присуството на ендемични, релектни и ретки растителни видови во Р. Македонија е од особено значење за науката. Во согласност со студентите што до сега се направени може да се заклучи дека е голем бројот на таквите видови (Мицевски, 1995; 1998). Позначајните релектни и ретки растителни видови во Република Македонија се прикажани во Табела 2.

Територијална поделба на ендемитите во Р. Македонија и проблемот со нивната загрозеност

Територијата на Р. Македонија е богата со ендемити, а тоа е условено со геолошкото минато на овој дел на Балканскиот Полуостров и неговата флорогенеза. За сега се смета дека има околу 111 локални ендемични видови и 24 во пограничните планини. Источниот дел на Македонија, источно од реката Вардар скоро и да нема ендемити, додека останата територија западно од Вардар е многу богата со вакви таксони. Од планините најбогата е Галичиница, од клатурите – клатурата на реката Треска, а од низините посебно се истакнува околината на Прилеп. Ендемитите во Македонија може да се групираат во 5 посебни групи (Micevski & Matevski, 1987). На многу мал дел од ендемитите во Р. Македонија им се заканува опасност од уништување.

Ендемизмот во флората во Македонија е во непосредна врска со геолошката историја, со климатските состојби во минатото и денес на оваа територија, кои се заслоелни за одреден планински масив или планински дел. Овој феномен некои поедини видови да се строги ендеми коишто се изкуствено врзани за тој дел или масив, дека е невозможно да преживее и да се одржи до денес. Скоро секој пого деен период на принос на старата терциерна флора успеал да преживее и да се одржи до денес. Скоро секој пого деен период на принос на старата терциерна флора успеал да преживее и да се одржи до денес. Скоро секој пого деен период на принос на старата терциерна флора успеал да преживее и да се одржи до денес. Скоро секој пого деен период на принос на старата терциерна флора успеал да преживее и да се одржи до денес. Скоро секој пого деен период на принос на старата терциерна флора успеал да преживее и да се одржи до денес. Скоро секој пого деен период на принос на старата терциерна флора успеал да преживее и да се одржи до денес. Скоро секој пого деен период на принос на старата терциерна флора успеал да преживее и да се одржи до денес. Скоро секој пого деен период на принос на старата терциерна флора успеал да преживее и да се одржи до денес. Скоро секој пого деен период на принос на старата терциерна флора успеал да преживее и да се одржи до денес. Скоро секој пого деен период на принос на старата терциерна флора успеал да преживее и да се одржи до денес. Скоро секој пого деен период на принос на старата терциерна флора успеал да преживее и да се одржи до денес. Скоро секој пого деен период на принос на старата терциерна флора успеал да преживее и да се одржи до денес. Скоро секој пого деен период на принос на старата терциерна флора успеал да преживее и да се одржи до денес. Скоро секој пого деен период на принос на старата терциерна флора успеал да преживее и да се одржи до денес. Скоро секој пого деен период на принос на старата терциерна флора успеал да преживее и да се одржи до денес. Скоро секој пого деен период на принос на старата терциерна флора успеал да преживее и да се одржи до денес. Скоро секој пого деен период на принос на старата терциерна флора успеал да преживее и да се одржи до денес. Скоро секој пого деен период на принос на старата терциерна флора успеал да преживее и да се одржи до денес. Скоро секој пого деен период на принос на старата терциерна флора успеал да преживее и да се одржи до денес. Скоро секој пogo деен период на принос на старата терциерна флора успеал да преживее и да се одржи до денес. Скоро секој пого деен период на принос на старата терциерна флора успеал да преживее и да се одржи до денес. Скоро секој пого деен период на принос на старата терциерна флора успеал да преживее и да се одржи до денес. Скоро секој пого деен период на принос на старата терциерна флора успеал да преживее и да се одржи до денес. Скоро секој пого деен период на принос на старата терциерна флора успеал да преживее и да се одржи до денес. Скоро секој пого деен период на принос на старата терциерна флора успеал да преживее и да се одржи до денес. Скоро секој пого д...
во Македонија, од кои некои се прилагодени на многу специфични услови во животната средина и се со многу ограничено распространување.

Иако има податоци за 135 ендемични видови, смета дека е суштина важно да се говори за точниот број на растителните ендемити, билејки деталните испитувања и монографската обработка на многу родови откриваат нови локални ендеми. Со интензивиране на таксономските и хоролошките истражувања, се случува и да се намалува бројот на ендемитите со откривање на нивни нови наоѓалки во другите региони или соедени земји. Така на пример познатото ендемито за соединени Албанија – *Colchicum pieperianum* Markgr. кој бил познат само за локалитетот Деја бил најден и на планината Бистра и денес синонимот на овој таксон е *Colchicum macedonicum* Kosanin.

Според расположивите податоци за нашата флора би можеле орентации да се наведат 111 локални ендемични видови, кои според сегашните познавања за нивниот опсег се со многу ограничено распространување и исклучиво се наоѓаат на територијата на Македонија. Покрај овие ендеми постои што еден број од 24 ендеми кои во Македонија се наоѓаат во нивното историско - геолошко минато успеале да се одржат до денес. Тука спаѓаат многу интересни видови: *Salvia jurisicii*, *Astragalus cernjavski*, *Tulipa marianae*, *Alyssum bargalense*, *Ferulago macedonica*, *Hedysarum macedonicum* и *Potentilla tridentula*, а на нив им се приключува и еден голем број на релативно нови видови кои кога нас единично наоѓаат на тој локалитет. Останатите видови кои се наоѓаат во клисури на Вардар воведено со распространети на денесната страна од реката. Во однос на групирането на ендемите односно нивната подделба разни автори како на пример Адамовиќ, Turrill, Гајиќ и др. имале различни погледи односно критериуми за формирање групи (Micevski & Matevski, 1987).

Поделба на ендемите според големината на просторот што го зафаќаат

Ендемите во Македонија се поделени врз основа на големината на просторот и областа што го зафаќаат (Micevski & Matevski, 1987). На тој начин се поделени во 5 групи:

- I група, опфаќа ендеми кои се многу ограничени и познати само од еден локалитет.
- II група опфаќа ендеми кои се пошироко распространети на територијата на Македонија.
- III група опфаќа ендеми кои се наоѓаат во западните погранични краеви.
- IV група опфаќа ендеми од јужните погранични планини.
- V група опфаќа ендеми на Балканското Полуостров.

I група ендемични растенија, ендеми само на еден локалитет

Од сите групи на ендеми најинтересен е групата кои завземаат многу мал и ограничен простор и често таа се видови кои се прилагодени на многу специфични услови на животната средина. Тоа се главно видови кои се наоѓаат на палеогенети седишта како на пример *Astragalus cernjavski* и *Tulipa marianae*, кои се познати само на еден локалитет едвојно од 0,5 km². Слично е и со видовите *Hedysarum macedonicum*, *Salvia jurisicii* и *Ferulago macedonica*, а на нив има значително ограничено распространување. Опсегот на распространувањето на овој таксон е значително мал и вариантот на популациите се ограничени во нивните нови наоѓалишта во другите региони или со усилјување на распространувањето нивото на нивната штета и се во поголема големина по своето потекло и состав.

Ако се анализира распоредот на ендемите на територијата на Македонија може да се појасни до значителен дел на нивното разпространување и се насочува и на значителен дел на нивното разпространување. Така, нивните видови кои се наоѓаат во нивните нови наоѓалишта во другите региони или со усилјување на распространувањето нивото на нивната штета и се во поголема големина по своето потекло и состав.

Во текот на истражувањата биле застапени многу интересни видови: *Verbascum* и *Centaurea* кое се наоѓаат на македонските пагови и на врвот на Дебар. Поради значителна градба на науката на нивната разпространување и на нивното историско - геолошко минато успеале да се претстават на првото стопанство на микробиологичките и хоролошките истражувања. Во минатото биле најден и на нивото на нивната разпространување и на нивното историско - геолошко минато успеале да се претстават на првото стопанство на микробиологичките и хоролошките истражувања. Во минатото биле најден и на нивото на нивната разпространување и на нивното историско - геолошко минато успеале да се претстават на првото стопанство на микробиологичките и хоролошките истражувања.
Фармакогностички интересни ендемични растенија во Република Македонија

Уште еден интересен случај е видот Astragalus psysocalyx кој бил описан 1837 година од Fischer, од околината на Пловдив во Бугарија. 50 години подоцна наоѓалиштето од кое е описан овој вид е целосно уништено и тој вид исчезнал. Подоцна бил пронајден во околината на Петрич. Во борбата против работење на природата бил удавачен и пошт е целосно уништен. Денес единствено наоѓалиште е во Македонија во околината на Гевгелија во областа со дабови (Quercus coccifera), каде што успешно расте и покажува доброт на човекот.

Кон овие видови може да се приклучат и уште некои кои ги населуваат закривните на поедин планини и завземаат мали површини како на пример Colchicum macedonicum (Бегово поле на Јакупица) и Pedicularis ferdinandi кои кои ги населуваат врвовите на поедин планини најчесто излегуваат и во Албанија и во Грција. Тоа бил излегуван во внатрешноста на Македонија и пограничните области.

Иако скоро сите високи планини во Македонија имаат свој локален ендемичен видови сепак две планини, Галиција и Шар Планина се одликуваат со богатство од ендеми. За Галиција се изведени 13 ендеми, а за Шар Планина 10. Шар Планина според својата положба и однос со другите планини претставува бариера која многу видови не успеале да ја преминат во текот на повеќекратните миграции на човекот и поради тоа се задржале на неа. И Галиција претставува сложен случај само со разликата во тоа што на неа се задржале видови што доаѓале од јужните грчки планини.

Напомена е дека кај овие планини настануваат процеси на еволуција на видовите на ендемичните растенија. Ендемите можат да претставуваат специфична група од видови кои се наоѓаат на определени планини со единствено видови на кои не се наоѓаат на други места. Ендемите можат да претставуваат специфична група од видови кои се наоѓаат на определени планини со единствено видови на кои не се наоѓаат на други места. Ендемите можат да претставуваат специфична група од видови кои се наоѓаат на определени планини со единствено видови на кои не се наоѓаат на други места. Ендемите можат да претставуваат специфична група од видови кои се наоѓаат на определени планини со единствено видови на кои не се наоѓаат на други места. Ендемите можат да претставуваат специфична група од видови кои се наоѓаат на определени планини со единствено видови на кои не се наоѓаат на други места. Ендемите можат да претставуваат специфична група од видови кои се наоѓаат на определени планини со единствено видови на кои не се наоѓаат на други места. Ендемите можат да претставуваат специфична група од видови кои се наоѓаат на определени планини со единствено видови на кои не се наоѓаат на други места. Ендемите можат да претставуваат специфична група од видови кои се наоѓаат на определени планини со единствено видови на кои не се наоѓаат на други места. Ендемите можат да претставуваат специфична група од видови кои се наоѓаат на определени планини со единствено видови на кои не се наоѓаат на други места. Ендемите можат да претставуваат специфична група од видови кои се наоѓаат на определени планини со единствено видови на кои не се наоѓаат на други места. Ендемите можат да претставуваат специфична група од видови кои се наоѓаат на определени планини со единствено видови на кои не се наоѓаат на други места. Ендемите можат да претставуваат специфична група од видови кои се наоѓаат на определени планини со единствено видови на кои не се наоѓаат на други места. Ендемите можат да претставуваат специфична група од видови кои се наоѓаат на определени планини со единствено видови на кои не се наоѓаат на други места. Ендемите можат да претставуваат специфична група од видови кои се наоѓаат на определени планини со единствено видови на кои не се наоѓаат на други места. Ендемите можат да претставуваат специфична група од видови кои се наоѓаат на определени планини со единствено видови на кои не се наоѓаат на други места. Ендемите можат да претставуваат специфична група од видови кои се наоѓаат на определени планини со единствено видови на кои не се наоѓаат на други места. Ендемите можат да претставуваат специфична група од видови кои се наоѓаат на определени планини со единствено видови на кои не се наоѓаат на други места. Ендемите можат да претставуваат специфична група од видови кои се наоѓаат на определени планини со единствено видови на кои не се наоѓаат на други места. Ендемите можат да претставуваат специфична група од видови кои се наоѓаат на определени планини со единствено видови на кои не се наоѓаат на други места. Ендемите можат да претставуваат специфична група од видови кои се наоѓаат на определени планини со единствено видови на кои не се наоѓаат на други места. Ендемите можат да претставуваат специфична група од видови кои се наоѓаат на определени планини со единствено видови на кои не се наоѓаат на други места. Ендемите можат да претставуваат специфична група од видови кои се наоѓаат на определени планини со единствено видови на кои не се наоѓаат на други места. Ендемите можат да претставуваат специфична група од видови кои се наоѓаат на определени планини со единствено видови на кои не се наоѓаат на други места. Ендемите можат да претставуваат специфична група од видови кои се наоѓаат на определени планини со единствено видови на кои не се наоѓаат на други места. Ендемите можат да претставуваат специфична група од видови кои се наоѓаат на определени планини со единствено видови на кои не се наоѓаат на други места. Ендемите можат да претставуваат специфична група од видови кои се наоѓаат на определени планини со единствено видови на кои не се наоѓаат на други места. Ендемите можат да претставуваат специфична група од видови кои се наоѓаат на определени планини со единствено видови на кои не се наоѓаат на други места. Ендемите можат да претставуваат специфична група од видови кои се наоѓаат на определени планини со единствено видови на кои не се наоѓаат на други места. Ендемите можат да претставуваат специфична група од видови кои се наоѓаат на опреде
 ámbа е клисурата на реката Треска која има 6 енде-
mii (Thymus skopjensis, Viola herzogii, Silene lindneri, Dianthus kapinensis, Thymus oehmiatus и Cerasium cernjavskii), потоа Таорската клисур со 4 ендеми (Anchusa macedonica, Tragopagon kindingeri, Hesperis macedonica и Sempervivum kindingeri), дознот тек
на Црна Река има 3 ендеми (Verbascum macedonicum, Verbascum herzogii и Cytisus lupinfolis), Демиркапис-
ката клисур има 2 ендеми (Heptaphtera macedonica и Centarea formanekii), додека во клисурите на реките Бабуна, Црн Дрим и Рајец има само по еден ендемит (Viola babunensis, Campanula deborensis и Verbascum chrysanthum). Во досегашната ботаничка литература многу често се пишувало за богатството на клисурите со ендеми, мегутоа се дошло до заклучок дека клисур-
слуѓитите местото на Прилеп и тоа еден е во непосредна близина на Црна Река има 3 ендеми (
на Црна Река има 3 ендеми (Verbascum macedonicum, Verbascum herzogii и Cytisus lupinfolis), Демиркапис-
kата клисур има 2 ендеми (Heptaphtera macedonica и Centarea formanekii), додека во клисурите на реките Бабуна, Црн Дрим и Рајец има само по еден ендемит (Viola babunensis, Campanula deborensis и Verbascum chrysanthum). Во досегашната ботаничка литература многу често се пишувало за богатството на клисурите со ендеми, мегутоа се дошло до заклучок дека клисурите се многу побогати со релелнит видови (Micevski & Matevski, 1987).

Ендеми во низините и ридските места

Третото место што е поврзано со ендемитите се низи

ните и ридските места. Во тој поглед посебно се знача-

ни три локалитети. Првите два локалитети се во око-

лината на Прилеп и тоа еден е во непосредна близина на градот. Тој локалитет се одликува со присуство на големи гранични блокови помеѓу кои растат 4 ендеми: Centaurea karamani, Verbascum adenanthurum, Moeringia minutiflora и Asplenium macedonicum. Вторниот локали-

tот го опфака теренот околу Плетвар со врвот Козјак-

ските ЗРП, а субендемитите (главно балкански енде-

мични видови (Aiii) се застапени во 62% од македон

ниот сектор. Седиштето на организацијата е во Гланд,

стотици партнери од невладини организации и приват

ата најголема еколошка организација во светот, корис

членуваат повеќе од 1 000 владини и невладини орга-

и за заштита на растенијата (Меловски и сор., 2010):

1. толкување и документирање на растителниот диверзитет (растителна разновидност),
2. заштита на растителниот диверзитет,
3. одржливо користење на растителниот диверзитет,
4. едукација на тема “Растителен диверзитет”,
5. зајакнување на капацитетите за заштита на растителниот диверзитет.

Потенцијални значајни растителни подрачја (ЗРП) во Македонија

Според наведените критериуми во Македонија се

издвоени 42 значајни растителни подрачја од кои 12 се

наоѓаат на границите со соседните земји. Според кри-

териумот А се класифицирани 40 ЗРП, а сите 42 се класи-

фицирани според критериумот С. Загрозените енде-

мични видови (Aiii) се застапени во 62% од македон-

ските ЗРП, а субендемитите (главно балкански енде-
Слика 1. Некои од типичните македонски ендемити се искористени како филателистички орнаменти

Таблица 3. Фармакогностички интересни ендемични растенија, со локалитети и класификацијата според IUCN (Меловски и сор., 2010)

<table>
<thead>
<tr>
<th>Вид</th>
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<th>Локалитети</th>
<th>IUCN</th>
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<td>Пелистер, Бабуна, Бистра, Галичица, Јакупица, Мариово, Прилеп, Скопска Црна Гора, Таорска клисура, Клисура на река Треска, Водно, Шар Планина</td>
<td>R</td>
</tr>
<tr>
<td>Alkanna nonneiformis</td>
<td>Boraginaceae</td>
<td>Мариово, Прилеп</td>
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<td>Armeria vandasii</td>
<td>Plumbaginaceae</td>
<td>Прилеп</td>
<td>R</td>
</tr>
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<td>Aspleniaceae</td>
<td>Мариово, Прилеп</td>
<td></td>
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<tr>
<td>Astragalus cernjavskii</td>
<td>Fabaceae</td>
<td>Криволак</td>
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<td>Алшар-Трибор</td>
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<td>Прилеп</td>
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<th>Компаративен вид од истог род</th>
<th>Фармакогностички значајни податоци на компаративниот вид</th>
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<td>Alchemilla vulgaris L.</td>
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<td>Asplenium trichomanes</td>
<td>Херба: токоферол, холестерол, ситостерол и стигмастерол, киселини, флавоноиди и др. Дејство: експекторант, еменагог и лаксатив.</td>
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<td>Centaurea cyanus L.</td>
<td>Цвет: флавоноиди и антоцијани, танини, кумарини, слузи и др. Дејство: намалува инфекција на очите, дејствува антиинфламаторно дејство и спречува создавање каменчиња во уринарниот тракт. Има и еменаго, антиинфламаторно и тонизирачко дејство, и се користи при габични инфекции, треска, кашлица, констипација, нарушувања во функцијата на црниот дроб и жолчката и други состојби (Takeda &amp; Tominaga, 1983; Garbacki et al., 1999).</td>
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<td>7.</td>
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<td>Dianthus superbus</td>
<td>Херба: антоцијанин, емодин, естри на бензоева киселина, ситостерол-3-О-глукозид, емодин-8-О-гликозид, изоориентин, фицион, тритерпенски сапонини и др. Дејство: диуретично (Li et al., 2000).</td>
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<td>9.</td>
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<td>Hedysarum polybotrys</td>
<td>Корен: танини, флавоноиди, сапонини, протеини и Јаглехидрати (Hai et al., 2004; Piccaglia et al., 2003). Дејство: антиоксидантно и на ацетилхолин естераза (Chen et al., 2007; Hailiqian et al., 2007).</td>
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Фармакогностички интересни ендемични растенија во Република Македонија

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<td>Salvia jurisicii Kossanin, Lamiaceae</td>
<td>Salvia officinalis L.</td>
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<td>17.</td>
<td>Sempervivum octopodes Turill, Crassulaceae</td>
<td>Sempervivum tectorum L., Sempervivum thopsonianum Wale.</td>
<td>Лист: полифеноли (кемпферол е единствен агликон), делфинидол (единствен антоцијанидин), 4-тиобензил (-)-епигалокатехин, 4-тиобензил (-) епигалокатехин-3-галат, слузни матери, смолести матери и др. Дејство: Атстрингентно, антиоксидантно, антимикробно асептично (Blázovics et al., 2003; Sentjurc et al., 2003).</td>
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<td>20.</td>
<td>Tulipa scardica Borrm., Lilaceae Verbascum herzogii Bornm., Verbascum macdonedicum Kosanin &amp; Murb., Viola allichiens Beck Viola arsenica Beck, Violaceae</td>
<td>Viola tricolor L.</td>
<td>Цвет: сини, глицирична киселина и незрнени деривати (метил естири и гликозиди), флавониони (виолантин и рутин), сапонини, алкалоиди, танини, слузни матери и смоли (Toiu et al., 2009; Vukics et al., 2008a; Molnár &amp; Szabolcs, 1999). Дејство: експекторантно, антиоксидантно, антивируспираторно и лаксативно (witkowska-Banaszczak et al., 2005; Vukics et al., 2008a; Molnár &amp; Szabolcs, 1999).</td>
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* цитатот се однесува на соодветен web-cite:
1 – Lousewort (Pedicularis muscicola). http://www.martindalesnutrition.com/ns/DisplayMonograph.asp?StoreID=le1jvdg81e92l70g03a0et9d9x0r89&DocID=bottomline-lousewort;

Макед. фарм. билт., 55 (1, 2) 41 - 55 (2009)
Табела 5. Фармакогностички интересни субендемични растенија според локалитети и класификацијата според IUCN (Меловски и сор., 2010)

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* Глобална црвена листа
Фармакогностички интересни ендемични видови во флората на Р. Македонија

Според определени податоци, 10% од светската флора се растенија што се декларираат како лековити, ароматични или медицински, но нивната комерцијална стойност е малена поради малите производи. Историски, ендемичните видови биле фармакогностички интересни за испитување на хемискиот состав на основу на нивната уникалност и потенцијална фармакологичка активност. Во однос на фармакогностички интересни ендемичните видови во флората на Р. Македонија, само видовите Sideritis raeseri и Sideritis scardica се чуваат како специфичен раст во рамките на нивната ендемичност. Во сите наведени видови, видовите Sideritis raeseri и Sideritis scardica биле фармакогностички интересни, а во класата на монокотиледони, има само 109 ендемички видови.
супстанции и испитување на биолошко-фармакологија активност. Секако дека новите инструментални техники што денес се користат во хемијата на медицинските растенија и хемијата на природните производи овозможуваат користење на минимално количество материјал, што не претставува ризик за природните популатија на ендемичните видови. Во поглед на биолошко-фармаколошката аналiza, bio-assay водените техники би овозможиле рационално користење на екстракти и изолатите од интерес, со што исто така би се нашло потреба од собирање поголема количество материјал. Дополнителен предизвик претставува секако планирање на соодветна програма за заштита на сите ендемични растенија без оглед на степенот на нивната загрозеност, со особен акцент на видовите што се фармакогностички интереси и што покажале позитивни результати во фармакогностичката аналiza.

Литература


Habel, J.K., Assmann, T., Relict Species: Phylogeography and Conservation Biology, Springer, Heidelberg. Available at: http://books.google.com/books?id=v-M TCbd.7gWUC&pg=PA100&lpg=PA100&dq=armeria+official+drug+medicinal&source=bl&ots=QaaFBKo812&sig=ENW45y5SB1s-Y-SeOe5i5OTEF&m-k=hl=en&ei=19mTe-HMo7AswbV1rnUCA&sa=X&oi=book_result&ct=resul t&resnum=6&ved=0CDwQ6AEwBQ#v=onepage&q&f=false


International Union for Conservation of Nature. Available at: http://www.iucn.org/about/


Nature protection and biodiversity FYR Macedonia, European Environment Agency.
Saxifraga granulata. Ayurvedic Herbs For UTI, Kidney
Maced. pharm. bull., 55 (1, 2) 41 - 55 (2009)
Pharmacognostically interesting endemic plant species in the flora of Republic of Macedonia

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Key words: endemic plant species, Republic of Macedonia, pharmacognosy

Flora in the Republic of Macedonia comprises about 3200 species in 147 families. According to some sources there are 115 endemic higher plants, of which, 114 belong to gymnosperm. According to other sources, there are 135 species of endemic plants and about 111 of which are local endemic species and 24 are stretched in the border mountains. The exact number has not been determined yet. Eastern part of Macedonia, east of the river Vardar almost poses no endemics, while the rest of the territory, west of the Vardar is very rich in such species. The richest areas with endemic plants are Galicica Mountain, Treska River Gorge and the lowlands surrounding the city of Prilep.

Despite the wealth of endemic and relict species, any pharmacognostical data for these plants have not been published yet. Of all these endemic species, 30 could be pharmacognostically interesting for future investigation of the chemical composition, isolation of potentially active substances and testing biological-pharmacological activity. Modern analytical techniques utilized in the examination of the chemistry of medicinal plants and natural products require a very small amount of material does not pose a risk of endangering endemic species. An additional challenge is the development of an appropriate program for the protection of all endemic, pharmacognostically interesting species.
Professional competences, credentialing and continuing professional development in the pharmacy profession
- Model Framework for Patient Centered Pharmaceutical Care -

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Abstract

The crucial changes have taken place in the role of pharmacy profession in the past decade. All these changes have been systematically evolved and adopted to support professional quality improvement aspect. In general, the worldwide professional and national authorities have been committed to develop the professional competencies, credentialing and continuing professional development; to emphasis the maintenance of high standards of professional development and in response to changes which occurred to pharmacy education and national health regulation policy. The constantly evolving health regulatory environment in each country will shape the progress of this process in the future.

This article provides a review of existing concepts for professional competences, credentialing and continuing professional development in pharmacy profession in an attempt to understand and clarify the complexity encountered in this comprehensive domain. It can also serve as a platform for the local interaction of a broad range of authorities in the health field.

Key words: pharmacy, competences, credentialing, continuing professional development, patient centered pharmaceutical care

Introduction

Drug therapy is the most frequently used form of treatment in any health practice setting. Namely, evidence-based practices confirm that drug therapy treatment has significantly increased due to the aging of world population, emerging of new infectious diseases, the prevalence of chronic diseases and increased number of patients who suffer from co-morbidities and require implementation of advanced multidrug therapy approach of complex nature (Kaplan and Laing, 2004). It must also be taken into account that community-based treatments for both acute and chronic illnesses that include newly developed therapies and act as the driving force towards outpatient surgeries have been related with the increased need of use of drugs. Moreover, with the development of the drug science and state-of-the art industrial technologies, the range of new efficient drugs has dramatically expanded. It is also worth to mention that the adoption and implementation of new health technologies created real opportunities for increased access to drugs. On the other hand, health technologies also alter the way of receiving health services and ultimately revolutionize healthcare operations. Nowadays, modern health facilities apply computerized dispensing techniques and devices. Since recently, drugs can also be obtained by mail order and via Internet. As far as the drugs are concerned, the increased number of prescriptions, and the increased number of available over-the-counter drugs (Fenichel, 2004), as a consequence focused the patient interest on self-directed care. The complexity of these features, as noted above, combined with these new circumstances have placed the pharmacist in a more prominent position in terms of providing more information and sophisticated services to patients. The new role of pharmacists requires improved platform of knowledge, skills, eth-
tical attitude, and behavior that would contribute to the development of competently trained and adequately credentialed professionals.

The objective of this general overview is to present the first initiative of competency and categories of credentialing of pharmacy professionals, continual education of pharmacists and the best practices, with special focus on patient centered pharmaceutical care framework. This concept, developed and established in USA, due to its sophisticated characteristics has served as the model for Europe and other regions. The parallel concepts have also been developed for pharmacy technicians. However, they are not the subject of this overview. This overview, also presents the different aspects of the professional development and pharmacist’s competencies that should be considered in our country, in the near future.

The process of achieving and maintaining the competency in the pharmacy profession and in the entire health care profession indeed, has been the subject of many support programs and initiatives which are to serve the public interest, healthcare professional oversight boards, pharmacy organizations, regulatory agencies, credentialing and governing boards (appendix A).

The basic principles underlying the roles and responsibilities of pharmacists are stated in the Code of Ethics for Pharmacists edited by American Pharmacists Association-APhA. (APhA, 2007). These principles, based on the moral obligations and qualities, are established in order to serve as a guidance for pharmacists in their relations with patients, health professionals, and society. Hence, defined basic principles include:

1. A pharmacist respects the covenantal relationship between the patient and pharmacist.
2. A pharmacist promotes the good of every patient in a caring, compassionate, and confidential manner.
3. A pharmacist respects the autonomy and dignity of each patient.
4. A pharmacist acts with honesty and integrity in professional relationships.
5. A pharmacist maintains professional competence.
6. A pharmacist respects the values and abilities of colleagues and other health professionals.
7. A pharmacist serves individual, community and societal needs.
8. A pharmacist seeks justice in the distribution of health resources

According to the fifth principle, it is obvious that professional competence is one of the key points in the pharmacy career.

The Institute of Medicine (IOM) identified five core competencies required for all health professionals (including pharmacists) aimed at optimizing patients’ outcomes. They are the following: (I) deliver patient-centered care; (II) work as part of an interdisciplinary team; (III) practice evidence-based medicine; (IV) apply quality improvement approaches; and (V) use information technology. These competencies are the base for developing relevant standards and competence statements concerning healthcare professionals. The recommendations targeting oversight organizations include integrating these core competencies into accreditation, and certification processes across the professions. IOM challenges health care oversight agencies (licensing boards and certifying agencies) to abandon reliance on continuing education in favor of a more systematic approach that require each practitioner’s competence be assessed, that interventions be targeted to specific deficiencies, and that each care-giver be tested to ensure that the desired competencies have been acquired and incorporated into practice. The employees must demonstrate professional judgment, ethics, attitudes, and values (Greiner and Knebel, 2003).

Citizen Advocacy Center (CAC) urges to put the subject of continual competence on the agendas of theirs and related organizations’ meetings and conferences to generate considerable interest and support. Thus, at CAC Conference held in June, 2000, the National Association of Boards of Pharmacy (NABP) representative stated that: “there has been a philosophical change in the way consumers approach health care practitioners”. It appeared that in the past, professionals used to say: “Trust me to be competent because I practice every day”. But, at present, patients are saying: “Demonstrate to me that you are competent because my life and well being are in your hands” (CAC, 2001). Moreover, CAC recommended a five-step framework for assessing and demonstrating continual professional competence: (i) routine periodic assessment, (ii) development of a personal improvement plan, (iii) implementation of the improvement plan documentation (steps for quality improvement) and (iv) demonstration of competence (quality assurance step). The critical first step is routine periodic assessment. It serves as the key to identify knowledge deficiencies requiring correction and to tailor lifelong learning1 choices according to the needs of individual health care professionals. Assessment also reveals whether a practitioner applies his or her knowledge and skills competently in clinical situations.

In addition, Council on Credentialing in Pharmacy (CCP) adopted the following definition about competence in 2000, as follow: “The ability to perform one’s duties accurately, make correct judgments, and interact appropriately with patients and with colleagues. Professional competence is characterized by good problem-solving and decision-making abilities, a strong knowledge base, and the ability to apply knowledge and experience to diverse patient-care situations” (CCP, 2006).

1 Lifelong learning includes all learning activities undertaken throughout life, with the aim of improving knowledge, skills and competences within a personal, civic, social and/or employment-related perspective (ECD,2003).

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It is more than clear that the pharmacists intend to take leadership roles in improving the use of medicines, which they cannot accomplish if working in isolation from the many others professionals involved in the medicines use process (WHO, 2006).

**Tasks and Functions of Pharmacists and Future Vision of Pharmacy Practice**

Contemporary pharmacy practice reflects an evolving pattern: from having a primary role in medicine distribution and advising patients to a much broader and team-based clinical role which includes provision of patient-centered medicine therapy management, health improvement, and disease prevention services. Thus, the pharmacy practice integrates fields that are directly related to the patient and performed in community pharmacy, ambulatory care clinics, hospitals, long-term care facilities, home-care institutions, and managed-care organizations.

However, other roles of pharmacists are practiced in the pharmaceutical industry, research and development, national agencies, academia, associations, and a number of unique healthcare practices such as drug and poison information centers that are not directly related to patient care.

The Model State Pharmacy Act and Model Rules of the NABP define the practice of pharmacy as follows: The “Practice of Pharmacy” means the interpretation, evaluation, and implementation of Medical Orders; the Dispensing of Prescription Drug Orders; participation in Drug and Device selection; Drug Administration; Drug Regimen Review; the Practice of Telepharmacy within and across state lines; Drug or Drug-related research; the provision of Patient Counseling; the provision of those acts or services necessary to provide Pharmacist Care in all areas of patient care, including Primary Care and Collaborative Pharmacy Practice; and the responsibility for Compounding and Labeling of Drugs and Devices (except Labeling by a Manufacturer, Re-packager, or Distributor of Non-Prescription Drugs and commercially packaged Legend Drugs and Devices), proper and safe storage of Drugs and Devices, and maintenance of required records.

The practice of pharmacy also includes continually optimizing patient safety and quality of services through effective use of emerging technologies and competency-based training (NABP, 2006).

Following the third Joint Commission of Pharmacy Practitioners (JCPP) – “Pharmacy in the 21st Century”, at the Conference in 1994, a collaborative effort of ten national pharmacy organizations led to the development of the Pharmacist Practice Activity Classification (PPAC), a hierarchical categorization of pharmacist activities (Table 1). The PPAC is focused primarily on activities of licensed, practicing pharmacists across the continuum of health care settings. The PPAC also includes activities that are either delegated by pharmacists to technicians or are carried out by automated systems. The PPAC facilitates the comparable data among studies, such as: building databases for statistical purposes about pharmacists’; patient-centered activities (to improve patient outcomes); the use of resources and provides a solid foundation to support systems for payment model that can be used for billing. The classification captures a range of activities from a traditional dispensing-based practice towards a higher level of patient care and direct patient care services.

This document classify pharmacy practice activities as follows: the highest level is the Domain or field of activity where four major domains of pharmacist activities have been identified. Within each domain there are more specific Classes of Activities. Within each Class there are Activities or Interventions - labels for sets of specific behaviors that, based on their professional knowledge and clinical judgment, pharmacists engage in as a part of their professional practice to enhance patient care and outcomes. Under many of the activities, one or more Tasks are specified. Some tasks are further divided into distinct Steps. Each entry has a unique alphanumeric identity. It is expected that this design will allow for easy and timely modification of the system (Maine, 1998)-Table 1.

**Table 1. Pharmacist Practice Activity Classification (PPAC); Domain and Classes related to practice activity**

<table>
<thead>
<tr>
<th>Domain</th>
<th>Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Ensuring Appropriate Therapy and Outcomes</td>
<td>A.1. Ensuring appropriate pharmacotherapy</td>
</tr>
<tr>
<td></td>
<td>A.2. Ensuring patient’s understanding/adherence to his or her treatment plan</td>
</tr>
<tr>
<td></td>
<td>A.3. Monitoring and reporting outcomes</td>
</tr>
<tr>
<td>B. Dispensing Medications and Devices</td>
<td>B.1. Processing the prescription or drug order</td>
</tr>
<tr>
<td></td>
<td>B.2. Preparing the pharmaceutical product</td>
</tr>
<tr>
<td></td>
<td>B.3. Delivering the medication or device</td>
</tr>
<tr>
<td>C. Health Promotion and Disease Prevention</td>
<td>C.1. Delivering clinical preventive services</td>
</tr>
<tr>
<td></td>
<td>C.2. Surveillance and reporting of public health issues</td>
</tr>
<tr>
<td></td>
<td>C.3. Promoting safe medication use in society</td>
</tr>
<tr>
<td>D. Health Systems Management</td>
<td>D.1. Managing the practice</td>
</tr>
<tr>
<td></td>
<td>D.2. Managing medications throughout the health system</td>
</tr>
<tr>
<td></td>
<td>D.3. Managing the use of medications within the health system</td>
</tr>
<tr>
<td></td>
<td>D.4. Participating in research activities</td>
</tr>
<tr>
<td></td>
<td>D.5. Engaging in interdisciplinary collaboration</td>
</tr>
</tbody>
</table>

According to the well known consensus document: “The Future Vision for Pharmacy Practice 2015” that has

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Pharmacists will benefit society and have the potential to improve patient care and public health outcomes. The concept of “optimal medication therapy” implies that the use of medicines occurs within a system that assures the highest possibility of achieving desired clinical, humanistic, and economic outcomes. The “JCPP Vision” further states that: “Pharmacists will benefit society and be essential to the provision of effective health care by ensuring that: (a) medication therapy management is readily available to all patients; (b) desired patient outcomes are more frequently achieved; (c) overuse, underuse, and misuse of medications are minimized; (d) medication-related public health goals are more effectively achieved; and (e) cost effectiveness of medication therapy is optimized” (JCPP, 2004).

With the aim of achieving the mission of pharmacy profession and fulfilling these professional activities, a sound pharmacists’ education and numerous post-graduate studies and training opportunities have been introduced and made available to pharmacists.

I. Professional competencies addressing pharmacy practice

1. Competency based education - credential needed to prepare for pharmacy practice

Miller, graded the competency-based education on four levels: the learner knows the facts (cognition), knows how to apply the facts, shows how (in a controlled environment) and does (behavior, in real situations) (Miller, 1990). At the beginning, the curricula lead the learner from dependent and directed towards independent, self-directed and lifelong learner. Pharmaceutical education needs to develop content and process of the educational curriculum that is required to prepare students to render pharmaceutical care at the entry points in the health care system.

As concerns the competency based education, accreditation standards and guidelines have been established for the Professional Program in Pharmacy leading to the Doctor of Pharmacy Degree by the Accreditation Council for Pharmacy Education-ACPE (ACPE, 2006). According to Standard No. 9, the Goal of the Curriculum is: “The college or school’s professional degree curriculum must equip the graduates with professional competencies in order to enter the pharmacy practice in any setting and ensure optimal medication therapy outcomes and patient safety, satisfy the educational requirements for obtaining a license as a pharmacist, and meet the requirements for the university degree. The curriculum must provide the graduates with knowledge that meets the criteria of good science; professional skills, attitudes, and values; and the ability to integrate and apply learning to both the present practice of pharmacy and the advancement of the profession. Graduates must be able to identify and implement needed changes in pharmacy practice and health care delivery”.

The AACP (American Association of Colleges of Pharmacy) proposed a series of initiatives under the Center for the Advancement of Pharmaceutical Education (CAPE) with the aim of supporting and facilitating the efforts of colleges and schools of pharmacy in the US for transforming their curricula and supporting the education of future practitioners to deliver pharmaceutical care. Important fea-

Table 2. Alignment of AACP CAPE educational outcomes and ACPE Standard No.12

<table>
<thead>
<tr>
<th>AACP CAPE Educational outcomes 2004</th>
<th>Outcome 1: Pharmaceutical care</th>
<th>Outcome 2: System managements</th>
<th>Outcome 3: Public health</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Provide patient-centered care</td>
<td>*Manage human, physical, medical, informational and technological resources</td>
<td>*Assure the availability of effective, quality health and disease prevention services</td>
<td>*Promote health improvement, wellness, and disease prevention in cooperation with:</td>
</tr>
<tr>
<td>*Provide population-based care</td>
<td>*Manage medication use systems</td>
<td>*Develop public health policy</td>
<td>• patients,</td>
</tr>
<tr>
<td>Provide patient care in cooperation with:</td>
<td>Manage and use resources of the health care system, in cooperation with:</td>
<td></td>
<td>• communities,</td>
</tr>
<tr>
<td>of an interprofessional health care team</td>
<td>• patients,</td>
<td>to promote health;</td>
<td>• at-risk populations, and</td>
</tr>
<tr>
<td>based upon sound therapeutic principles and evidence-based data, taking into account relevant legal, ethical, social, cultural, economic, and professional issues, emerging technologies, and evolving biomedical, pharmaceutical, social/behavioral/administrative, and clinical sciences that may impact therapeutic outcomes.</td>
<td>• prescribers,</td>
<td>to provide, assess, and coordinate safe, accurate, and time-sensitive medication distribution; and</td>
<td>• other members of an interprofessional team of health care providers.</td>
</tr>
</tbody>
</table>

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Assure Safe and Effective Pharmacotherapy and Optimize Therapeutic Outcomes

Assure safe and Accurate Preparation and Dispensing of Medications

Table 3

<table>
<thead>
<tr>
<th>Area 1</th>
<th>Assure Safe and Effective Pharmacotherapy and Optimize Therapeutic Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area 2</td>
<td>Assure safe and Accurate Preparation and Dispensing of Medications</td>
</tr>
<tr>
<td>Area 3</td>
<td>Provide Health Care Information and Promote Public Health</td>
</tr>
</tbody>
</table>

There is no doubt that AACP CAPE Educational Outcomes 2004, ACPE Standard No.12 Professional Competencies and Outcome Expectations, NAPLEX® Blueprint and the PPAC are closely related to the five core competencies applicable to all healthcare professionals and recently identified by the IOM.

However, NAPLEX should not be considered as the final examination of the college’s or school’s curricula. Therefore, in 2005, NABP introduced the Pharmacists Self-Assessment Mechanism (PSAM) (NABP, 2005). The PSAM is 100 questions assessment for pharmacists to evaluate their professional practice skills and knowledge. The PSAM Blueprint Competencies are similar to those of NAPLEX. Moreover, the MPJE (Multistate Pharmacy Jurisprudence Examination), combines federal and state-specific questions to test the pharmacy jurisprudence knowledge of prospective pharmacist. It serves as the pharmacy law examination in participating jurisdictions. Graduates from foreign colleges of pharmacy must document that they have education and experience equivalent to their US-trained colleagues. They must also pass the Foreign Pharmacy Graduate Equivalency Examination (FPGEE), the Test of English as a Foreign Language (TOEFL), and the Test of Spoken English (TSE). Once these requirements are met, they receive the Foreign Pharmacy Graduate Examination Committee’s (FPGEC) certificate. In addition to the NAPLEX® and MPJE®, some US states require a laboratory examination or an oral examination before licensure is conferred.
All state boards also require that candidates complete an internship before being licensed. The internship may be completed during the candidate’s academic training or after graduation, depending on the state requirements. An internship requirement traditionally requires a pharmacy student to work in a licensed pharmacy under the supervision of a board-registered pharmacist or to complete college-coordinated clerkships as equivalent to (or as a significant portion of) state-mandated internships: A license to practice Pharmacy as a Pharmacy Intern shall be granted only to those individuals who: (1) are enrolled in a professional degree program of a school or college of pharmacy that has been approved by the Board and satisfactorily progressing toward meeting the requirements for licensure as a Pharmacist; or (2) are graduates of an approved professional degree program of a school or college of Pharmacy or are graduates who have established educational equivalency by obtaining a Foreign Pharmacy Graduate Examination Committee™ (FPGEC®) Certificate, who are currently licensed by the Board of Pharmacy for the purpose of obtaining practical experience as a requirement for licensure as a Pharmacist; or (3) are qualified applicants awaiting examination for licensure or meeting Board requirements for re-licensure; or (4) are participating in a residency or fellowship program.

**Licensure renewal:** licensure renewal is mandatory for pharmacists who wish to continue to practice their profession. All member state boards of pharmacy require that registered pharmacists complete a minimum number of hours or continual education units (CEUs) before they can renew their licenses. Continual education is defined by CCP as: Organized learning experiences and activities in which pharmacists engage after they have completed their entry-level academic education and training. These experiences are designed to promote the continuous development of the skills, attitudes, and knowledge needed to maintain proficiency, provide quality service products, respond to patient needs, and keep abreast of change (CCP, 2001).

More recently (June 2003), ACPE adopted the following definition: Continuing education for the profession of pharmacy is a structured process of education designed or intended to support the continuous development of pharmacists to maintain and enhance their professional competence. Continuing education should promote problem-solving and critical thinking and be applicable to the practice of pharmacy (ACPE, 2003).

The candidate for licensure renewal may acquire a number of CEUs by attending educational seminars, teleconferences, meetings, reading journal articles, completing home study courses or computer-based educational programs (CCP, 2006).

The hours or CEUs must be earned either through participation in a continuing education (CE) program whose provider has been accredited by the ACPE, or through a program or activity, which has been otherwise approved by the state board. Achievement of a satisfactory score on an assessment that is created by and submitted to the CE provider is generally required as a documentation of completion of a CE program.

The ACPE has established accreditation standards for providers of continuing pharmacy education. The majority of ACPE-approved providers are professional pharmacy organizations, colleges of pharmacy, and pharmaceutical companies. Each program is reviewed every 6 years by the ACPE. ACPE re-evaluates the CE model in pharmacy through a process of identification of the CE requirements of other organizations, exploration of the CE processes and activities of other health professions, domestic and international, including the use of new models, such as CPD. Also, ACPE is exploring the re-engineering of the CE provider accreditation process to make it more efficient and effective, while fostering continuous quality improvement and encouraging innovation (ACPE, 2001).

**II. Professional development and enhanced competency of pharmacist**

While, academic degrees in the field of pharmacy, state licensure and re-licensure are obligated, mandatory, other credentials (postgraduate degrees, certificates and certification), pharmacists earn to document their specialized or advanced knowledge and skills on voluntary bases. Pharmacy practitioners who have completed programs of various types that are intended to develop and enhance their knowledge and skills or those who have successfully documented a specialized level of knowledge and skills through an assessment process are awarded appropriate qualifications—Many different organizations (public and private) are directly involved in assessing pharmacists’ knowledge and skills, granting credentials and certificates, and accrediting educational programs and institutions. Post-licensure training programs and credentials are competency-based, developed on the basis of a comprehensive practice analysis in the relevant areas, and offered or accredited by an organization that adheres to the accepted standards and practices to assure quality, integrity, and validity. A pharmacist’s credentials are indicators that he or she holds the qualifications needed to practice the profession of pharmacy and is therefore worthy of the trust of patients, of other health care professionals and of the society as a whole (CCP, 2006; Scope of Contemp. PP, 2009).

The three categories of pharmacist credentials and oversight bodies are illustrated in Figure 1.

1. **Academic Postgraduate Education and Training**

Postgraduate master’s (M.S.) programs cover common fields of studies: business administration, clinical pharmacy and public health.

Postgraduate doctor of philosophy (Ph.D.) programs cover common fields of studies: pharmacology, pharmaceutics, pharmaceutical and medicinal chemistry, pharma-
Professional competences, credentialing and continuing professional development in the pharmacy profession

cotherapeutics, pharmacy practice and social and administrative sciences.

2. Residency Training program (Credential acquired: Residency Certificate)

A residency is an organized, directed postgraduate program, accredited by American Society of Health-System Pharmacists (ASHP) independently or in collaboration with other pharmacy organizations in a defined area of pharmacy practice. Residencies usually last 12 months, although certain specialized residencies require additional 12 (or continuous 24) months for completion - Table 4.

Pharmacy practice residencies - PGY1 (Post Graduate Year one)

Pharmacy practice residencies focus on the development of the resident of professional competence in the delivery of patient care (providing optimum medication therapy outcomes) and practice management activities (managing medication use process). This program provides an environment and structure for accelerating the growth and experience beyond entry-level professional competence through supervised practice under the guidance of model practitioners in “real-world” settings (hospital, community pharmacy, managed care organization, home or long term care practice). Residents are exposed to a wide range of patients with multiple diseases, chronic or acute, and work with a variety of health professionals, thereby advancing their clinical, interpersonal, and leadership skills.

Specialized pharmacy practice residencies PGY2 (Post Graduate Year two)

Specialized pharmacy practice residencies focus on the knowledge, skills, attitudes and abilities to raise the resident’s level of expertise needed to provide care in a specialized area of pharmacy practice (e.g., critical care, drug information, pharmacotherapy, or oncology). This specialized residency training is an organized, directed, accredited program that builds upon the competencies established in pharmacy practice residencies, after the first year of residency training. The second year of postgraduate residency training involves additional education and pharmacists obtain more in-depth training and experience. In partnership with the APhA for Community Pharmacy, and the Academy of Managed Care Pharmacy (AMCP) for Managed Care, the American Society of Health-System Pharmacists’ Research and Foundation has established residency standards and reviews for programs that include community and managed care pharmacy residencies. The National Association of Chain Drug Stores (NACDS) and National Community Pharmacists Association (NCPA) sup-

Figure 1. Pharmacy Credentials and Oversight Bodies for Pharmacists in U.S.
ported community pharmacy residency programs, including development of the NACDS/NCPA community pharmacy residency guidelines. The Institute for the Advancement of Community Pharmacy, (an organization founded by the NACDS and NCPA), has provided grants to encourage schools of pharmacy and community pharmacies to develop additional community pharmacy residency programs nationwide (Sheaffer, 2004).

3. Fellowships (Credential acquired: Fellowship Certificate)

Fellowship is direct, highly individualized postgraduate program that prepares the participant to become an independent researcher in an area of pharmacy practice. Fellowship programs are developed by faculties of pharmacy, academic health centers, universities, pharmaceutical manufacturers and usually last one to two years. ACCP (American College of Clinical Pharmacy) has developed guidelines for organization of clinical fellowships. To improve the consistency in the quantity and quality of the research experience, the ACCP has implemented a process for peer review of pharmacy fellowship training programs. If the specialist qualifications and the training program meet the guidelines based on a review by the ACCP Fellowship Review Committee, they are recognized and the program and specialist are listed on the ACCP Web site (CCP, 2006). Otherwise, there is no formal accreditation process.

4. Certificate Programs (Credential earned: Certificate of Completion)

A certificate program is a structured and systematic postgraduate continuing education experience for pharmacists that is smaller in magnitude and shorter in duration than degree programs. Certificate programs are offered by national and state pharmacy organizations and by schools and colleges of pharmacy and other educational groups (CCP, 2006). The design of certificate programs includes didactic instruction, practice experiences, simulations, and/or other opportunities for the demonstration of desired professional competencies. Attributes that differentiate certificate program from CE programs are practice experiences, simulations and other activities for demonstration of stated competencies. The length of any particular certificate program is determined by its stated goals, desired professional competencies and outcome measures. This generally requires a minimum of 15 contact hours (1.5 CEUs). Certificate programs are designed to instill, expand, or enhance practice competencies through the systematic acquisition of specified knowledge, skills, attitudes, and behaviors. For example, the APhA offers programs in such areas as asthma, diabetes, immunization delivery, and management of dyslipidemias. The value of these programs depends on individuals’ goals or in instances when an employer or regulatory body recognizes the importance of the certificate. Examples are state boards of pharmacy that allow pharmacists to administer vaccines, after they have completed a certificate program in vaccine administration.

5. Traineeships

In contrast to certificate programs, traineeships allow practicing pharmacists abbreviated clinical training experience through intensive, individualized, structured postgraduate programs (combination of self-study and didac-

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**Table 4. Residency Programs**

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<tr>
<th>Pharmacy practice</th>
<th>Specialized Residencies</th>
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<tr>
<td>• Pharmacy practice residency (traditionally conducted in health systems)</td>
<td>• Cardiology,</td>
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<td>• Pharmacy practice with emphasis on community pharmacy</td>
<td>• Clinical pharmacokinetics,</td>
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<td>• Pharmacy practice with an emphasis on managed care</td>
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<td>• Pharmacy practice with an emphasis on home care</td>
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<td>National Institute for Standards for Pharmacist Credentialing (NISPC)</td>
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<td></td>
<td>National Certification Board for Anticoagulation Providers (NCBAP)</td>
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<td>National Institute for Standards for Pharmacist Credentialing (NISPC)</td>
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<td>National Asthma Educator Certification Board (NAECB)</td>
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<td>National Institute for Standards for Pharmacist Credentialing (NISPC)</td>
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<td>National Certification Board for Diabetes Educators (NCBDE)</td>
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<td>American Board of Applied Toxicology</td>
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tic instruction) under supervision of a pharmacist practic-
ing in a given specialty. These programs provide the par-
ticipants with the knowledge and skills needed to give a
high level of care to patients with various chronic dis-
esees and conditions. Traineeships are generally of longer
duration (about five days) and involve smaller groups of
trainees than certificate programs. Examples of such pro-
grams are anticoagulation management, critical care phar-
macy, and diabetes management. These programs are com-
monly offered through the foundations of professional or-
izations such as the American College of Apothecar-
ies, (ACA), American Society of Consultant Pharmacists
(ASCP), and ASHP and are often supported by grants
from the pharmaceutical industry.

6. Certification

Certification is “voluntary process by which a non-
governmental agency or an association grants recognition
to an individual who has met certain predetermined qualifi-
cations specified by that organization”. The credentials are
granted to pharmacists and other health professionals who
have demonstrated a level of competence in a well-defined,
specific and relatively narrow area of practice. Certifica-
tion is granted on the basis of successful completion of rigor-
ously developed eligibility criteria that include a written
examination and, in some cases, an experiential compo-
ent. Also, certification usually requires initial assessment
and periodic reassessments of the individual’s knowledge,
skills and/or experience. The certification process is under-
taken and overseen by the Board of Pharmaceutical Spe-
cialties (BPS), the Commission on Certification in Geriat-
ric Pharmacy (CCGP), and the National Institute for Stan-
dards in Pharmacist Credentialing (NISPC).

I. Specialty certification (Credential acquired: Certifica-
tion in area of practice).

Pharmacists are certificated in five specialties by the
BPS: (1) nuclear pharmacy; (2) nutrition support pharma-
cy; (3) oncology pharmacy; (4) pharmacotherapy and (5)
psychiatric pharmacy. Later on, in 1997, BPS introduced
the designation of “Added Qualifications” to denote that
an individual has demonstrated an enhanced level of train-
ing and experience in one segment of a BPS-recognized

![Diagram](image-url)

**Figure 2.** Practitioners in direct patient care.
professional competences, credentialing and continuing professional development in the pharmacy profession

specialty. Infectious Diseases and Cardiology are the two added qualifications for the pharmacotherapy specialty currently recognized by BPS. Pharmacists who wish to retain BPS certification must be recertified every seven years.

II. Non-specialty certification

For example, to become certified by CCGP, candidates are expected to be knowledgeable about principles of geriatric pharmacotherapy and the provision of pharmaceutical care to the elderly. Pharmacists who meet CCGP’s requirements are entitled to use the designation Certified Geriatric Pharmacist, or CGP. Pharmacists who wish to retain their CGP credential must recertify every five years by successfully completing a written examination.

III. Disease management certification

NISPC offers certification in the management of diabetes, asthma, dyslipidemia, and anticoagulation therapy. To be certified by the NISPC, a pharmacist must pass an examination with questions that are specific to the specialty area, developed by experts, and designed to address four different areas of competency expected by all pharmacists who provide disease state management services to patients. There are no practice experiences or clinical training requirements in the specialty area. After passing the exam, pharmacists may use the designation of certified disease manager (CDM). Recertification is required every 3 years and is based on completion of 30 hours of CE in the specific disease state.

Also, multidisciplinary certification programs are available to professionals from many health disciplines, including pharmacists. Areas in which such certification is available include diabetes education, anticoagulation therapy, pain management, and asthma education. Table 5 shows post-licensure certifications and where they typically apply to pharmacists in narrowly focused and/or advanced areas of practice.

Relationship between the scope of a pharmacist’s practice and credentials and post-licensure education and training

Figures bellow (Figures 2, 3 and 4) present a framework for credentialing in pharmacy and summarize the elements (CCP, 2009). The framework attempts to illustrate: (1) how a pharmacist’s career may evolve or progress after completion of initial professional education, licensure, and entry to practice; (2) the post-graduate education and training activities and certifications undertaken by pharmacists; and (3) the correlations between credentialing, broad competency areas, scope of practice, and patient populations served. Figures 2, 3 and 4 deal only with the patient care domain, corresponding with AACP CAPE Education-

Figure 3. Post-licensure education and training relative to pharmacy practice.

Макед. фарм. билт., 55 (1, 2) 57 - 74 (2009)
al Outcome #1. However, Outcomes #2 and #3 are not included in the schematic presentations (CCP, 2009).

The surface A describes the practice of the community and hospital pharmacists. Surfaces B, C, or D reflect pharmacists professional development in a specific way. For example, pharmacists who choose to narrow their patient or practice focus (e.g. in diabetes or geriatrics) will move to surface B; pharmacists who elect to work with a broad base of patients and diseases, but also wish to substantially advance their level of knowledge, skills, and experience will move to surface C. An example of a pharmacist in this quadrant would be a pharmacotherapy specialist. Pharmacists in surface D have both narrowed their patient/practice focus and substantially advanced their knowledge and skills. An example of an Advanced Focused Practitioner would be a Board Certified Oncology Pharmacist (BCOP), one of the recognized specialty credentials in the pharmacy profession.

Figure 3 illustrates the range of post-licensure education and training activities pharmacists engage in to maintain their professional competencies and to support their continuing professional development.

Pharmacy practice residencies-(PGY1) provide training for generalists in hospitals, health systems, managed care, or community settings; hence their illustration is in Quadrant A in Figure 3. Specialized pharmacy practice residencies-(PGY2) residencies provide advanced training in a focused area of patient care. Traineeships, on the other hand, are more focused and would typically be undertaken by pharmacists with a narrower patient/practice focus (Quadrant B). Certificate Programs, which focus on the development of professional skills and their application in practice, would typically be undertaken by pharmacists in Quadrants A and B.

### III. Continuing Professional Development (CPD)

The Institute of Personnel and Development (IPD, UK) launched an early definition of CPD in October 1997: CPD is systematic, ongoing, self-directed learning. It is an approach or process, which should be a normal part of how you plan and manage your whole working life. Of note, the definition of CPD adopted by the National Health Service (NHS) in Great Britain, in 1999, makes reference to patients and healthcare outcomes:

*CPD is a process of lifelong learning for all individuals and teams of individuals which meets the needs of patients and delivers the health outcomes and healthcare priorities of the NHS and which enables professionals to expand to fulfill their potential.*

In 2002, the concept of CPD was described by FIP as:

*The responsibility of individual pharmacists for systematic*

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**Figure 4.** Post-licensure certifications and where they typically apply to pharmacists in narrowly focused and/or advanced areas of practice.
maintenance, development and broadening of knowledge, skills and attitudes, to ensure continuing competence as a professional, throughout their careers (FIP, 2002).

The same year, at a conference on lifelong learning, the following definition was offered:

Postgraduate professional education involving a cycle by which individual practitioners assess their learning needs, create a personal learning plan, implement the plan, and evaluate the effectiveness of the education intervention as it applies to their pharmacy practice (Hanson, 2002).

ACPE recent statement regarding CPD is that the CPD model provides the opportunity for quality improvement of the current system of continuing education, by building on the existing strong foundation of quality-assured, accredited continuing education for pharmacists (ACPE, 2003). The JCPE supports the concept of strong commitment to develop and maintain standards and programs to assure the public, governmental agencies, major employers and other influential organizations that pharmacists would maintain appropriate competencies throughout their careers.

The NABP in NABP RESOLUTION NO.99-7-03 TITLE (NABP, 2003): Continuing Pharmacy Practice Competency resolved that NABP endorse and encourage structured programs of continuing professional development. Hence, NABP encourage colleges, faculties, and schools of pharmacy and boards to collaborate on providing seminars to further pharmacist continuing professional development; the boards of pharmacy encourage, endorse, and support the efforts of NABP, the ACPE, and the AACP to instill and perpetuate the concepts of continuing professional development in students and pharmacists.

The AACP supports the concept of CPD, so AACP work actively with ACPE and other pharmacy organizations in exploring methods for facilitating its use within pharmacy (AACP, 2003).

All these definitions and statements clearly indicate that pharmacists have an ethical obligation and responsibility for their own lifelong learning, and the maintenance of the knowledge, skills, attitudes and abilities necessary to deliver professional services in line with accepted, contemporary professional standards and public expectations. Since the system of mandatory CE has shown that it does not provide a satisfactory degree of assurance that pharmacists are maintaining the level of competence adequate to meet public needs and expectations, the framework of CPD has been evolved as an agenda for lifelong learning.

However, CPD does not replace CE, but quality-assured CE is an essential component of CPD.

The need for CPD can be shortly summarized as follows:

- To ensure that pharmacists maintain (at an appropriate level) their knowledge, skills and competence to practice throughout their careers in their own specific (or current) area of practice;
- To improve the pharmacist’s personal performance (i.e., develop knowledge and skills);
- To enhance the pharmacist’s career progression

Besides, CPD is based on principles as follows (Picton and Brackley, 1999)-

- CPD is a systematic, ongoing cyclical process of self-directed learning;
- It includes everything that practitioners learn, which enables them to be more effective as professionals;
- CPD includes the entire scope of the practitioner’s practice, and may include activities both within and outside the usual work setting;
- CPD is a partnership between the practitioner and his/her organization, meeting the needs of both;
- The practitioner is responsible for his/her own professional development. The organization has a responsibility to help the practitioner meet the development needs that relate to performance in his/her current job

When considered together with the NHS definition given earlier, three important features of CPD are clear: CPD is practitioner-centered and self-directed; CPD is designed to be practice-related; CPD is outcomes-oriented in terms of maintaining competence, the professional development of the practitioner, meeting individual and organizational goals, and achieving improved patient outcomes. CPD has been described using four-stage and five-stage cycles (Figure 5).

Figure 5. CPD process source CPP 2004.

Reflect: referred to as “self-appraisal” or “assessment,” this stage entails the pharmacist reflecting on personal and organization needs and goals for professional development, and self-assessing his/her knowledge, skills and competence. Reflection is important to learning; it has been defined as the complex and deliberate process of thinking about and interpreting an experience in order to learn from it. (Boud, Keogh and Walker, 1985).

Plan: involves the design of a personal development plan (PDP). The plan includes all the activities that will address the identified learning and development needs and goals. The outcomes should be linked to one or more spe-
cific professional competencies (ACPE, 2003). The plan could include structured programs (such as accredited CE), as well as a diverse range of informal learning activities, many of which will be work-based or work related (Figure 6).

Figure 6. The PDP is recorded in the personal portfolio. Each pharmacist’s situation is unique, so no two sets of learning needs and personal plans will be the same.

Act: putting the plan into action is the next well known stage.
Evaluation: can be carried out by the individual practitioner, by the practitioner’s peers, or by the practitioner’s supervisor or manager. In some CPD models (for example, in the UK), the portfolio is subject to review by the regulatory body. For example, in Ontario, Canada there is the opportunity for small-group peer review of the learning portfolio, and also for direct assessment of knowledge and skills. Some form of third-party review or evaluation of the portfolio would appear to be valuable, not only to provide feedback to the pharmacist, but also as a means to identify those that may be experiencing difficulty in one or more aspects of CPD, and in need of assistance or remediation, and to protect society from the few practitioners who otherwise would not self-assess and correct deficiencies. Feedback from third parties should be given in a constructive and non-threatening way, with the primary objective being to help the individual move forward in his/her professional development.

Record: central to the CPD cycle is the practitioner’s personal portfolio, which becomes a comprehensive record, like a professional diary or transcript covering all the stages. The portfolio, which can be electronic or paper-based, should be readily accessible, and simple to use. Ideally, a standardized format should be adopted to facilitate training, data entry and, where applicable, portfolio evaluation. In CPD portfolios, pharmacists record all relevant learning experiences (accredited-CE or informal work-based) or work related activities. In time, the pharmacist’s portfolio will develop into a comprehensive record of education and practice with multiple possible applications.

CPD is based on the above mentioned principles and adopts educational strategies that have proven to be effective. It potentially offers a quality improvement to the current systems for pharmacist CE. While an appropriate, competency-based education can prepare a pharmacist to enter practice, no professional program can provide or develop all the aspects of the knowledge, skills, attitudes and abilities that a pharmacist will ever need. These require a combination of an appropriate pre-service educational foundation, in-service training, hands-on work experience, and lifelong learning. For professionals, there is no doubt that education is a continuum. As acknowledged, the educational strategies, and the competency and outcomes based approach that are successfully utilized for pre-service training must be maintained throughout the practitioner’s career. For all above mentioned, the state boards of pharmacy are requested, and expected, to protect the public by ensuring, through regulation, that licensed pharmacists are competent to deliver pharmacy services, as professionals.

Conclusion

As anticipated, due to the dynamic and intensely developing healthcare environment in the past several years, the pharmacist’s role has dramatically changed from that of conventional compounder and dispenser to one of “drug therapy manager”. The latter comprehensive role of the pharmacist involves spectrum of new responsibilities and best practice services to ensure that wherever the drug therapy is concerned, the best quality products to be selected, procured, stored, distributed, dispensed and properly administered in a manner so as to contribute to the health of patients with the risk reduced to the minimum for the patient. Now, the scope of pharmacy practice is more focused on patient-centered care, with all the cognitive functions of counseling, providing drug information and specific issues related to the managing and monitoring drug therapy. Pharmacists need to maintain their professional competence throughout their careers in order to provide safe, effective and quality professional services to patients and achieve the most positive patient outcomes possible. Schools of pharmacy prepare their graduates to acquire the necessary and rational competencies to enter practice, but the ongoing professional program can also provide or develop knowledge, skills, attitudes and abilities that a pharmacist will need in practice. Only a combination of an appropriate educational foundation, in-service training, hands-on work experience, lifelong learning, training and ongoing licensure, certification and evaluation of competencies will assure professional competence. Particularly noteworthy aspect about continuing professional development is that it can further engage pharmacists as adult learners, and enhance the overall effectiveness and outcomes of continuing education. Creating models for professional development provides the opportunity for quality improvement of the current system of continuing education, building on the existing strong foundation of quality-assured, accredited continuing education for pharmacists.

Health care systems, more stringent regulations and the existing oversight programs of licensure and certification agencies have the obligation to assure the public of the
safety and quality of health care, including the pharmaceutical care. It is of paramount importance and great responsibility of each individual pharmacist to determine whether a higher standard is required in any area relating to their individual professional practice. And, finally, pharmacists as health care professionals have to commit themselves to lifelong learning, in order to remain current and proficient as the science base for medicine continues to evolve and become more complex.

There is no doubt, that the models of professional competences, credentialing and continuing professional development in pharmacy exist in many worldwide countries intending to promote consistency and uniformity in the delivery of professional services. However, the US model deserves special attention owing to its comprehensive approach and advanced practical solutions.

Appendix

**AACP** American Association of Colleges of Pharmacy

AACP is a national organization representing pharmaceutical education in the United States. Their mission is to represent and advocate for all segments of the pharmaceutical academic community.

[www.aacp.org](http://www.aacp.org)

**AAPT** American Association of Pharmacy Technicians

AAPT provides continuing education and services to help technicians update skills. They also represent member’s interests to the public as well as health care organizations.

[www.pharmacytechnician.com](http://www.pharmacytechnician.com)

**ACA** American College of Apothecaries

ACA is a research and education resource center that provides pharmacist with therapeutic information and other issues affecting the pharmacy profession. They also provide an inquiry support line, specialty practice education program, pharmacy-related publications, and current events in health care.

[www.acainfo.org](http://www.acainfo.org)

**ACCP** American College of Clinical Pharmacy

ACCP provides pharmacists the leadership, education, and other resources needed in clinical practice and research. They support and promote research training and educational programs in pharmacotherapy.

[www.accp.com](http://www.accp.com)

**AFPE** American Foundation of Pharmaceutical Education

This foundation supports pharmacists to further their studies in advanced pharmaceutical science in industry, association work, academia, and other areas of professional practice. This foundation also provides high standards in education in colleges of pharmacy and American pharmacy through the support of ACPE, special programs of the AACP, and other key projects.

[www.afpenet.org](http://www.afpenet.org)

**AMCP** Academy of Managed Care Pharmacy

AMCP is a professional society, dedicated to promote the development and application of pharmaceutical care, to ensure appropriate health care outcomes for all patients. This association also provides for its members the leadership, and support in managed care.

[www.amcp.org](http://www.amcp.org)

**APhA** American Pharmacists Association

This association provides professional information and education for pharmacists. It also advocates a pharmacist to improve healthcare of patients through the provision of comprehensive pharmaceutical care.

[www.pharmacy.org](http://www.pharmacy.org)

**ASAP** American Society for Automation in Pharmacy

This organization aids its members in applying computer technology into pharmacy. Members include independent pharmacies, hospital pharmacies, colleges of pharmacy, state boards of pharmacy, state and national associations and government agencies.

[www.asapnet.org](http://www.asapnet.org)

**ASCP** American Society of Consultant Pharmacists

ASCP is an international pharmacy association for consultant pharmacists specializing in senior care. The association provides for it members leadership, education and resources needed for the practice of pharmacy in senior care.

[www.ascp.com](http://www.ascp.com)

**ASHP** American Society of Health-System Pharmacists

ASHP is a professional association that represents pharmacists who practice in hospitals, health maintenance organizations, long-term care facilities, home care, and other components of health care systems. Their main goal is to assist pharmacists to make the best use of medicine.

[www.ashp.org](http://www.ashp.org)

**ASP** Academy of Students of Pharmacy

ASP is the student section of APhA and it represents pharmacy and pre-pharmacy students in the United States and Puerto Rico. Its mission is to be the voice of pharmacy students and to prepare them to be professionals who provide and promote pharmaceutical care.

[www.pharmacy.org](http://www.pharmacy.org)

**ASPEN** American Society for Parenteral and Enteral Nutrition

ASPEN is a professional organization whose members are involved in the provision of clinical nutrition therapies, including parenteral and enteral nutrition. It prepares standards and guidelines for the use of nutrition support and...
professional practice. This agency also works with other nutrition, health care organizations, government agencies and insurance providers to offer patients the optimal use of nutrition therapies.

www.nutritioncare.org

ASPL American Society for Pharmacy Law
The purpose of this organization is to further the legal knowledge of pharmacists, students of pharmacy, students of law, attorneys, government, and other professions interested in legal issues affecting pharmacy and medication related issues. The agency also communicates accurate legal information to attorneys and pharmacists, educates pharmacists to their rights, distributes information, and provides forums.

www.aspl.org

BPS Board of Pharmaceutical Specialties
This organization trains and certifies pharmacists in a specialized field. Fields such as nuclear pharmacy, nutrition support pharmacy, oncology pharmacy, pharmacotherapy, and psychiatric pharmacy.

www.bpsweb.org

CCCP Canadian College of Clinical Pharmacy
http://www.cccp.ca/

CCGP Commission for Certification in Geriatric Pharmacy
CCGP is a national certification program for pharmacists who want to specialize in geriatric pharmacy practice. They are also responsible for establishing eligibility criteria to take the Certification Examination in Geriatric Pharmacy and establishing program policies.

www.ccgp.org

CCP Council of Credentialing in Pharmacy
This organization provides leadership, standards, public information, and coordination for the profession’s voluntary credentialing programs. Their goal is to provide credentialing programs in pharmacy that meet the established standards and quality.

www.pharmacycredentialing.org/default.htm

CPF Community Pharmacy Foundation
CPF is an organization with a primary purpose to assist community pharmacists by encouraging and fostering improvements in patient care. They also support efforts of pharmacist intervention in achieving targeted therapeutic goals.

www.tcpf.org

CPNP College of Psychiatric and Neurologic Pharmacists
CPNP is a professional membership association that represents pharmacists involved in the pharmaceutical care of psychiatric and neurologic patients. CPNP’s main goal is to assist pharmacists as they work to apply evidence-based, cost efficient best practices in achieving patient recovery and improved quality of life. www.cpn.org

FIP International Pharmaceutical Federation
This organization represents both pharmacists and pharmaceutical scientists worldwide. Its main purpose is to educate and the development of the practice and science of pharmacy.

www.fip.org

IACP Institute for the Advancement of Community Pharmacy
This institute supports educational initiatives, research projects and programs to enhance community pharmacy practice in the United States. IACP also promotes the value of community pharmacists and pharmacies

www.advancepharmacy.org

JCPP Joint Commission of Pharmacy Practitioners
JCPP was established to serve as a discussion forum for the CEOs and elected presidents of all major national pharmacy practitioner organizations. There are full members (AMCP, ACA, ACCP, APhA, ASCP, ASHP, and NCPA) and liaison members (AACP, ACPE, NABP, and NCSPAE). JCPP meets four times per annum.

NABP National Association of Boards of Pharmacy
NABP is an association that is committed in enforcing uniform standards, jurisdictions and assisting board members nationally and internationally. This association spans from the United States, Guam, Puerto Rico, New Zealand, eight Canadian Provinces and four Australian states.

www.nabp.net

NACDS National Association of Chain Drug Stores
The chief purpose of this association is to represent the views and policy positions of member chain drug companies. This is accomplished by various programs, services, and issues that the association is involved in.

www.nacds.org

NCPA National Community Pharmacists Association
This association represents pharmacist owners, managers, and employees of nearly 250,000 independent community pharmacies across the United States. Their goal is to represent the professional and proprietary interests of independent community pharmacists.

www.ncpanet.org

NCPDP National Council for Prescription Drug Programs
This organization’s goal is to create and promote data interchange standards in pharmacy industry, provide information and resources to educate industry, and support the needs of their members. NCPDP brings together diverse leaders of industry and decision-makers to their annual
Professional competences, credentialing and continuing professional development in the pharmacy profession

NCPAE National Council of State Pharmacy Association Executives
This association represents each state’s organization in providing business and professional development material, continuing education for pharmacists, pharmacy students, and pharmacy technicians and various other membership services.
www.ncpae.org

NIPCO National Institute for Pharmacist Care Outcomes
The national accrediting organization for pharmacist care education and training programs leading to the pharmacist care Diplomate credential.
www.nipco.org

NPhA National Pharmaceutical Association
The purpose of this organization is to represent the interests and needs of minority pharmacists in all practice settings. NPhA is also interested in advancing the standards of pharmaceutical care among all pharmacists.
www.npha.net

NPRT National Pharmacists Response Team
This organization purpose is to prepare pharmacists, pharmacy students, and pharmacy technicians that are interested in counteracting any possible terrorist attacks. Individuals trained will be called upon to assist in a mass vaccination or chemoprophylaxis campaign.
www.aphanet.org/pharmcare/NPRTPage.htm

NPTA National Pharmacy Technician Association
NPTA is an organization for pharmacy technicians. Its stated mission is to help enhance, promote, and enrich the lives and careers of every pharmacy technician. Its vision is to provide unmatched education and support for pharmacy technicians around the world.
www.pharmacytechnician.org

PhRMA Pharmaceutical Research and Manufacturers of America
This organization represents the country’s leading research-based pharmaceutical and biotechnology companies. It also supports young scientists in the pharmaceutical industry by awarding them with fellowships and grants at critical decision points of their career.
www.phrma.org

PTCB Pharmacy Technician Certification Board
PTCB is a nonprofit organization that oversees the certification program of pharmacy technicians in all practice settings. This organization develops the education and the exams for certification.
www.ptcb.org

PTEC Pharmacy Technician Educators Council
PTEC is an association representing pharmacy technician educators. Its primary mission is to assist the profession of pharmacy in preparing high quality well-trained technical personnel through education and practical training.
www.rxptec.org

SNPhA Student National Pharmaceutical Association
SNPhA was founded in 1972 as an extension of NPhA to pharmacy students. This association is educates and services students concerned about pharmacy services, professional development, and the lack of minority representation in pharmacy and other health related professions.
www.snpha.com

References
Резиме

Професионални компетенции, квалификации и континуиран професионален развој во фармацевтската професија
- Модел рамка за фармацевтска грижа насочена кон пациент -

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1 Јавна Институција во Здравствен сектор, за потребите на ПХИ Универзитетски Клиники, Институти и Ургентен Центар, Скопје

Ключни зборови: фармацевтска професија, компетенции, квалификации, континуиран професионален развој, фармацевтска грижа насочена кон пациент

Во текот на изминатата декада, улогата на фармацевтската професија претрпува суштински промени. Сите промени се развивани и усвојувани на еден систематичен начин од аспект на унапредување на квалитетот на професијата. Воопшто, професионалните и националните авторитети, ширум светот, се посветени на развојот на професионалните компетенции, квалификации и континуираниот професионален развој за да се нагласи одржување на високи стандарди за професионален развој и како одговор на промените што се случуваат во фармацевтска едукација и националните здравствени политики. Постојаното унапредување во контекст на здравствената регулатива за секоја земја, во иднина, ќе го обликува прогресот на овој процес.

Овој труд дава преглед на постојеци концепти за професионалните компетенции, квалификации и континуиран професионален развој во фармацевтската професија со цел да се вочи и објасни комплексноста присутна во овој опсежен домен.
Testing for drug and alcohol abuse at the workplace

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Abstract

Drug and alcohol abuse in the workplace represents a great risk to employee’s health and safety. More than 50% of the employees worldwide are related to easily accessible drug abuse, while 70% of the employees are related to alcohol abuse in the workplace. Tests for detecting drug and alcohol abuse in the workplace should be part of a new regulation, compulsory for all employees in the Republic of Macedonia. Implementing this sort of testing program should at the same time be a step towards devising particular solutions that shall bring about greater safety in the working environment. A key element in the implementation is to devise and establish an adequate policy that shall determine the risk factors within a working establishment which shall clearly express its position regarding drug and alcohol abuse during working hours. Along with the risk factors, the policy may also include the program for testing both, employees and the ones who are about to be employed, for drug and alcohol abuse. In order to implement this sort of test, it must be in accordance with the Occupational Safety and Health Act (Official gazette of the Republic of Macedonia, No 92/07, 2007) and a legal framework has to be defined, that shall regulate and solve numerous aspects of this issue, in order to fully implement the program for drug free working environment pursuant to the Declaration and the decrees of the United Nations General Assembly in 1998.

Key words: drugs and alcohol abuse, workplace, employees, drugs and alcohol testing.

Introduction

Drugs and alcohol are a plausible and definite risk for the employees’ safety in the work environment (Ghodse, 2005). The abuse of alcohol and drugs can occur in any workplace. According to the statistics more than 50% of employees worldwide are connected with misuse of readily available drugs and 70% of them with alcohol abuse in the workplace (Bennett, Lehman, 2000). The abuse of alcohol and other drugs may damage both, the physical and the mental health (Commission for Occupational Safety and Health Act, MIAC, 2008). The impairment of behavior can result in increased risk of injury or harm (Drug & Alcohol Information Centre, 2007) and this kind of abuse can also affect employees’ productivity, safety and security, decision making, morale, as well as the organizational image and the community relations (Breugem et al., 2006).

In the Republic of Macedonia testing for drug and alcohol abuse in the workplace should be a new legal regulation, mandatory for all employees. The introduction of the drug testing program should at the same time be an introduction to the process of solution making that will signify greater safety in the workplace. The Poison Control Center (PCC) (together with the toxicological laboratory) should be the carrier of the activities related to drug testing at the workplace as well as in developing new programs for testing, control and education that can help employers cope with this kind of issues.

Considerations before conducting a drug testing program

The first step in the risk management process is identifying hazards and hazards factors. When assessing whether alcohol and other drug use poses a safety and health hazard at the workplace, a range of factors should be considered. At some workplaces, the hazards associated with al-
cohol and other drugs may be greater due to the nature of the workplace. Hazards or hazard factors that are important to consider in relation to increased risk of injury or harm if workers are impaired by alcohol or other drugs include operation of machinery, driving in the course of work, situations where concentrations or motor coordination is relied on to carry out a job, use of hazardous substances and performing duties as part of a team. Even when people return a zero alcohol or drug level they may still be impaired by the “hangover” effects that can last beyond the direct presence of the drug and create risks. The hazards and risks associated with alcohol and drug use at the workplace should be assessed in the same way as other occupational safety and health issues (Work Cover Corporation of South Australia, 2006).

According to the Occupational Safety and Health Act, (Official gazette of the Republic of Macedonia, No. 92/07, 2007), and according to other supporting regulations there is no specific reference to alcohol and other drugs. The solution is all parties at the workplace to comply with their general “duty of care” in relation to usage of alcohol and other drugs and their potential acute and chronic effects regarding safety and health in the workplace. Also there are other legislations that a relevant to alcohol and drugs but to keep in mind that these legislations are closely connected to a specific social and working area (Work Cover Corporation of South Australia, 2006). In order to conduct a drug testing and to achieve a drug-free workplace, employers must develop drug-free workplace program. A comprehensive drug-free workplace program generally includes developing a drug-free workplace policy. The primary aim of the policy is to provide a clear documented guide regarding the workplace’s stance on drug and alcohol issues in relation to the workplace and to define the role of the employees, supervisors and in dealing with alcohol and drugs related work issues (Hunter Centre for Health Advancement, 2000). The program also includes supervisor training, employee education, employee assistance and drug testing. Employers may choose not to include all five components but it is recommended to explore all of them while developing a drug-free workplace program. Research shows that more components may lead to a more effective program. However, because every business is unique, there is not only one way to establish a drug-free workplace program. In order to conduct a drug testing it is necessary to coordinate this component with the Occupational Safety and Health Act in which no specific provisions are related to alcohol and drug testing. As a result employers are enabled to choose whether to test if risk assessments show particular risk, unless their organization is subjected to certain federal laws (ex. transportation drug-testing regulations, or aviation drug-testing regulations and others), as well as to keep in mind that industries may have industry-specific legislations or codes that deal with alcohol and other drugs at the workplace and these should be referred to, and also the drug testing is a contentious area (Work Cover Corporation of South Australia, 2006). It is recommended for the employer before conducting any drug-testing program to have a written policy that clearly outlines the necessity for the drug-testing. Therefore employers should carry out risk assessment in order to establish the nature of the policies and programs according to the level of risk at the workplace.

Testing for drugs and alcohol abuse at the workplace

Drug and alcohol abuse in the workplace is an issue that poses great threat to employees health, safety and security. That is why analysis is essential. According to the Occupational Safety and Health Act (Official gazette of the Republic of Macedonia, No. 2/07, 2007), employers are obliged to undertake necessary measures regarding the health and safety of the employees. By doing this, the potential and specific dangers may be identified, resulting in taking proper actions for their on time elimination, isolation, as well as minimization of their influence.

The Poison Control Centre (PCC) may take part in developing certain measures and activities in order to help employers interested in the development and improvement of the programs for testing drug and alcohol abuse in the workplace, by organizing educational courses, workshops and providing advice regarding the benefits and the significance of the health and safety effects of these analysis in both, the working and the living environment.

For great deal of employers, the drug testing program as well as the alcohol testing program may bring about less absence from work, decline in team changes when working in shifts, improvement of the health, safety and morale of the employees, as well as increase in work efficiency and productivity.

What is to be tested

After conducting the drug-testing program different procedures can be carried out in order to test alcohols, cocaine, cannabinoids, designer drugs, prescribed medications not used for medical purposes (such as opiate analgesics, sedative hypnotics), inhalants, hallucinogens, narcotics and also active substances within the drug which have significant effect on CNS. The material that is to be tested includes different kinds of samples, preferably urine, saliva and hair which are less painful to collect as well as blood samples which represent a bit more painful procedure.

Group of people to be tested and when to be tested

The drug and alcohol testing in the workplace can be carried out not only on all new applicants before being employed in the service, but on the already employed ones as well. This is of great significance for employees in a workplace with high risk of injury or illness, as well as other...
job positions defined as high-risk and sensitive in terms of health and safety of the employees, their associates and customers. These job positions include the organic-chemical and oil industry, construction, aviation, tourism, transportation of passengers, technical and health care, police and military structures, working with dangerous and harmful chemicals, explosives, etc. The drug and alcohol abuse testing program shall be implemented in the following cases:

**Pre-employment testing:** drug and alcohol abuse affects the person’s behavior, psychophysical ability, and work efficiency;

**Post accident/incident testing:** employees that have been directly involved in certain incidents must immediately undertake testing in order to discover whether the drug and/or alcohol abuse, as well as medicament abuse (with significant effect on the CNS) are the factors that affected the accident;

**Intentional testing:** employees are being tested due to suspicion of drug and/or alcohol abuse;

**Periodic testing or random testing:** all employees or a certain group of employees working at a high risk and safety-sensitive job position are being tested randomly, without being given previous notice. The testing may include:
- random selection of employees from a certain team; or
- testing each employee within a team, randomly selected, several times in a defined period of time.

Also, the testing may be performed on people (such as: suspended workers) who are involved in rehabilitation programs, once again by means of random choice in 12 to 24 months, in order to obtain better monitoring of the therapy efficiency. The decision for performing periodic testing on employees must be in accordance with the protection of privacy and human rights.

**Current position of the testing for drugs and alcohol abuse at the workplace in other countries**

According to other countries’ stance (USA, Canada, Australia), the introduction of the alcohol and drug testing should be made in consultation with employees, an Occupational Health and Safety (OHS) representatives and union representatives. The drug testing can be introduced if a risk assessment has identified that there are risks involved in undertaking certain activities whilst under the influence of alcohol and other drugs. Privacy, confidentiality and the legal position of employees and management should also be considered. The alcohol and other drug testing should be implemented as part of a comprehensive alcohol and drug program with appropriate safeguards, clear policy and procedures, and provision of education and counseling. The alcohol and drug testing in the workplace should also be introduced if there are existing legislative provisions, such as those relating to rail safety workers, passenger transport workers and heavy vehicle drivers. There is also legislation

prohibiting employees from working while intoxicated in the mining and aviation industries (Commission for Occupational Safety and Health Act, MIAC, 2008).

**Current position of the testing for drugs and alcohol abuse at the workplace in the Republic of Macedonia**

Currently, in the Republic of Macedonia the programs for drug and alcohol testing among employees are still a legal challenge. In order to implement a drug-free workplace program and be able to conduct testing for drugs and alcohol abuse at the workplace, it is necessary to establish a legal frame that covers many aspects. This can be achieved by imposing regulation that enables:
- implementation of the new safety strategy in the work environment;
- monitoring danger of hazards;
- conducting prevention, education, rehabilitation;
- clear definition of the rights and accountability of employees and employers;

Also it is very important to set rulebooks for proper and methodological collection of samples for detecting drugs and alcohol presence in urine and blood as well as to set rulebook for establishing referential values of some drugs and their metabolites in blood, urine, saliva hair etc.

**Conclusion**

A constructive step for employers to address alcohol and other drugs safety and health issues is to develop a workplace alcohol and other drugs policy, with supporting procedures, which address specific circumstances at the workplace. The development of a written policy and supporting procedures provides an opportunity to develop a range of management strategies designed to deal with issues that could arise. One important strategy for preventing problems is to provide information, education and training to all people at the workplace about the effects of alcohol and other drugs and their risks to safety and health, and the alcohol and other drugs policy and supporting procedures if developed. Providing information about alcohol and other drugs also contributes to the development of a workplace culture where workers are aware of the potential risks to safety and health and are prepared to encourage each other to work safely.

**References**


Резиме

Испитување на злоупотребата на дроги и алкохол на работното место

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Ключни зборови: злоупотреба на дроги и алкохол, работно место, вработени, тестирање на дроги и алкохол.

Злоупотребата на дрогите и алкохолот на работното место претставува голем риск по здравјето и безбедноста на вработените. Повеќе од 50% од вработените во светски рамки се поврзуваат со злоупотреба на лесно достапни дроги, а 70% од нив со злоупотреба на алкохол на работното место. Испитувањата за злоупотреба на дрогите и алкохолот во работната средина треба да биде нова законска обврска, задолжителна за сите вработени во Република Македонија.

Воведувањето на програмата за испитување на оваа проблематика треба истовремено да биде и вовед во креирањето на одредени решенија кои ќе значат поголема безбедност во работната средина. Клучен елемент претставува креирањето и воспоставувањето на соодветна полиса каде преку утврдување на ризик факторите во работната установа јасно ќе се дефинираат нејзините ставови во однос на злоупотребата на дроги или алкохол во текот на работното време. Согласно ризик факторите, во полисата може да биде вклучена и програмата за испитување на дроги и алкохол кај вработените, како и кај лица кои допрва треба да се вработат. За да се спроведе ваквото испитување мора да постои усогласување со Законот за безбедност и здравје при работа (Службен весник на РМ, бр. 92/07, 2007) и истовремено да се изготви и дефинира правна рамка со која ќе се регулираат и решат многу аспекти од оваа проблематика, со цел да се имплементира програмата за работна средина без дроги согласно Декларацијата и заложбите на Генералното собрание на Организацијата на Обединетите Нации (ОН) од 1998 година.
INSTRUCTIONS FOR AUTHORS

Macedonian Pharmaceutical Bulletin is an official publication of the Macedonian Pharmaceutical Association. The journal publishes original scientific papers, short communications, reviews, mini-reviews and professional papers from all fields of pharmacy and corresponding scientific fields of interest for pharmacy (pharmaceutical and medicinal chemistry, immunology and immunochemistry, molecular biology, pharmaceutical analyses, drug quality control, pharmaceutical technology, pharmacoinformatics, pharmacoconomics, biopharmacy, pharmacology, applied botany, pharmacognosy, toxicology, clinical pharmacy, food and nutrition, physical pharmacy, organical synthesis, social pharmacy, history of pharmacy etc.).

The Macedonian Pharmaceutical Bulletin also publishes and other contributions (recommendations and announcements, reports of meetings, important events and dates, book reviews, various rubrics).

Types of paper

Original scientific papers (full length manuscripts) should contain own unpublished results of completed original scientific research.

Short communications also should contain completed but briefly presented results of original scientific research. The article should be prepared as described for full length manuscripts, except for the following: the number of pages should not exceed 10 (including 2 illustrations, figures or tables). An Abstract should be included as well as a full reference list.

Reviews and mini-reviews are written at the invitation of the Editorial Board. “Mini-reviews” of a topic are especially welcome.

They should be surveys of the investigations and knowledge of several authors in a given research area, the competency of the authors of the reviews being assured by their own published results.

Professional papers report on useful practical results which are not original but help the results of the original scientific research to be adopted into practical use. Professional papers might be based on the elaborating of theoretical data.

Language

Original scientific papers, short communications, reviews and mini-reviews should be written in good English (American or British usage is accepted, but not a mixture of these), while professional papers and all other contributions may be submitted in Macedonian.

Submission declaration

Submission of an article implies that the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language.

Policy and ethics

The work described in your article must have been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans http://www.wma.net/en/30publications/10policies/b3/index.html;


Uniform Requirements for manuscripts submitted to Biomedical journals http://www.icmje.org. This must be stated at an appropriate point in the article.

Submission

Please submit the manuscript electronically (e-mail address: magl@ff.ukim.edu.mk) as a single PDF file, which will be used in the peer-review process. All correspondence, including notification of the Editor’s decision and requests for revision, takes place by e-mail removing the need for a paper trail.
Referees

Please submit, with the manuscript, the names, addresses and e-mail addresses of 3 potential referees. Note that the editor retains the sole right to decide whether or not the suggested reviewers are used.

Papers received by the Editorial Board are sent to referees. The suggestions/comments of the referees and Editorial Board are sent to the author(s) for further action. The revised article should be returned to the Editorial Board as soon as possible but in no more than 30 days.

Preparation of manuscripts

Use of wordprocessing software

It is important that the file be saved in the native format of the wordprocessor used. The text should be typed (1½ spaced) on A4 paper with margins of 3.0 cm on each side in single-column format, font Times New Roman, Mac C Times, Macedonian Times and size 11, Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. In particular, do not use the wordprocessor’s options to justify text or to hyphenate words. However, do use bold face, italics, subscripts, superscripts etc. When preparing tables, if you are using a table grid, use only one grid for each individual table and not a grid for each row. If no grid is used, use tabs, not spaces, to align columns. The electronic text should be prepared in a way very similar to that of conventional manuscripts. To avoid unnecessary errors you are strongly advised to use the “spell-check” and “grammar-check” functions of your wordprocessor.

The pages in the article should be numbered.

Finally, please create PDF file before sending the article. After acceptance, you will be asked to supply the article as wordprocessing document (zip-file).

Appendices

If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

Abbreviations

Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

Units

Follow internationally accepted rules and conventions: use the international system of units (SI). If other units are mentioned, please give their equivalent in SI.

The names of substances should be in accordance with the IUPAC recommendations and rules or Chemical Abstracts practice.

Math formulae

Present simple formulae in the line of normal text where possible and use the solidus (/) instead of a horizontal line for small fractional terms, e.g., X/Y. In principle, variables are to be presented in italics.

Footnotes

Footnotes should be used sparingly. Number them consecutively throughout the article, using superscript Arabic numbers. Many wordprocessors build footnotes into the text, and this feature may be used. Should this not be the case, indicate the position of footnotes in the text and present the footnotes themselves separately at the end of the article. Do not include footnotes in the Reference list.

Table footnotes

Indicate each footnote in a table with a superscript lowercase letter.

Figures

Figures (photographs, diagrams and sketches) and structural formulae should each be given on a separate sheet (the place to which they belong in the text should be indicated). The figures should be numbered in Arabic numerals (e.g. Fig. 1). Ensure that each illustration has a caption. Supply all captions separately, not attached to the figure. A caption should comprise a brief title (not on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

Please submit the pictures in a black and white version.

Tables

The tables should be numbered in Arabic numerals (e.g. Table 1) and each should be given on a separate sheet (the place to which they belong in the text should be indicated). Number tables consecutively in accordance with their appearance in the text. Place footnotes to tables below the table body and indicate them with superscript lowercase letters. Be sparing in the use of tables and ensure that the data presented in the tables are not duplicated elsewhere in the article.
Instruction for Authors

Article structure

Manuscript should contain: title, abstract, key words, introduction, material and methods, results and discussion, conclusion, acknowledgment (if desired) references and summary.

Subdivision

Divide your article into clearly defined sections (Abstract, Introduction, Material and methods, etc.). Any section or subsection may be given a brief heading. Each heading should appear on its own separate line.

Essential title page information

Papers should be preceded by a title page comprising: the title, the complete name(s) of the authors, and the author’s affiliations.

Title. Concise and informative. Avoid abbreviations and formulae where possible.

Author names and affiliations. Where the family name may be ambiguous (e.g., a double name), please indicate this clearly. Present the authors’ affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript arabic number immediately after the author’s name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name of each author.

Corresponding author. Clearly indicate (with *) who will handle correspondence at all stages of refereeing and publication, also post-publication. Ensure that telephone and fax numbers (with country and area code) are provided in addition to the e-mail address and the complete postal address.

Each paper must begin with an Abstract which should not exceed more than 250 (original scientific and professional papers) or 100 (short communications) words. The abstract should state briefly the purpose of the research, the principal results and major conclusions. References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself. Immediately after the abstract, provide a list of 3 to 6 keywords arranged in the order according to their importance.

Introduction

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods

Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described. Manuscripts which are related to theoretical studies, instead of Material and methods, should contain a sub-heading and the Theoretical background where the necessary details for verifying the results obtained should be stated.

Results

Results should be clear and concise.

Discussion

This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Conclusions

The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Acknowledgements

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

References

Citation in text

Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either “Unpublished results” or “Personal communication”. Citation of a reference as “in press” implies that the item has been accepted for publication and a copy of the title page of the relevant article must be submitted.

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As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

Макед. фарм. билт., 56 (1, 2) 79 - 82 (2010)
Reference style

Text: All citations in the text should refer to:
1. Single author: the author’s name (without initials, unless there is ambiguity) and the year of publication;
2. Two authors: both authors’ names and the year of publication;
3. Three or more authors: first author’s name followed by “et al.” and the year of publication.
Citations may be made directly (or parenthetically).
Groups of references should be listed first alphabetically, then chronologically.
Examples: “as demonstrated (Allan, 1996a, 1996b, 1999; Allan and Jones, 1995). Kramer et al. (2000) have recently shown....”
List: References should be arranged first alphabetically and then further sorted chronologically if necessary.
More than one reference from the same author(s) in the same year must be identified by the letters “a”, “b”, “c”, etc., placed after the year of publication.
Examples:
Reference to a journal publication:
Reference to a book:
Reference to a chapter in an edited book:
Journal abbreviations source
Journal names should be abbreviated according to Index Medicus journal abbreviations: http://www.nlm.nih.gov/tsd/serials/lji.html
List of serial title word abbreviations: http://www.issn.org/2-22661-LTWA-online.php;
Manuscripts written in English should contain a Summary in Macedonian at the end of the paper. The summary should contain: title, author(s) full-name(s), surname(s), author’s affiliations (institution and address), key words and abstract. Professional papers written in Macedonian should contain a summary in English in which the same data should be included.

Submission checklist

It is hoped that this list will be useful during the final checking of an article prior to sending it to the journal’s
Editor for review. Please consult this Guide for Authors for further details of any item.

Ensure that the following items are present:
One Author designated as corresponding Author:
- E-mail address
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- All necessary files have been uploaded
- Keywords
- All figure captions
- All tables (including title, description, footnotes)
- Further considerations: Manuscript has been “spellchecked” and “grammar-checked”
- References are in the correct format for this journal
- All references mentioned in the Reference list are cited in the text, and vice versa
- Permission has been obtained for use of copyrighted material from other sources (including the Web)

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