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RESEARCH ON SOME PLANT SPECIES CONTAINING ESSENTIAL OILS PERFORMED AT UNIVERSITY OF MEDICINE AND PHARMACY „IULIU HAŢIEGANU" CLUJ-NAPOCA

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Abstract: The present article offers a synthesis of original research performed at the department of Pharmaceutical botany of „Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca on 17 species and varieties of essential oil-containing plants that belong to 11 genera, all Romanian traditional medicinal, endemic or ornamental species. The essential oils were qualitatively analized and quantified by TLC and GC-MS from: *Tanacetum balsamita* (2 varieties), *Artemisia abrotanum*, *Rhododendron myrtifolium, Origanum vulgare, Artemisia annua, Inula helenium*, *Salvia officinalis*, *Thuja occidentalis, Acorus calamus, Achillea* (4 species) and *Solidago* (3 species). The chemical composition of these species belonging to the Romanian flora was established, two chemical infraspecific taxa were identified within *Tanacetum balsamita* and *Achillea distans*, and toxic compounds from essential oils (β-asarone, thujone) were quantified.

Key words: essential oils, medicinal plants, chemical infraspecific taxa.

Introduction

The essential oils are odorant secondary plant metabolites produced and stored by specialized cells and tissues. Compared to fatty oils that leave traces on paper or other substrates, the essential oils evaporate without trace. They represent complex mixtures of numerous substances of great diversity, a few thousand being identified up to the present in different species. The main constituents are mono- and sesquiterpenes, aromatic compounds and phenyl-propanes. The terpenes can be hydrocarbons or their oxygen-containing derivatives (oxides, alcohols, aldehydes, ketons, acides, ethers, esters) or glycosides. The main plant families containing essential oils are in the pinophytes and magnoliophytes: Pinaceae, Cupressaceae, Myrtaceae, Lauraceae, Apiaceae, Rutaceae, Lamiaceae, Asteraceae, Zingiberaceae. In order to extract the essential oils from fresh or dried plant material, several techniques are available: extraction using fatty substances or organic solvents, cold-pressed extraction, steam and hydro-distillation, or more recently ‚green' methods such as ultrasound-assisted extraction, microwave-assisted extraction, sub- and supercritical fluid extraction, pressurized fluid extraction, pulsed electric fields, high voltage electrical discharges. In plants, their role seems to be protection against pathogens, or repellents, as well as the inter-relation with biological agents: attracting pollinators or as alelopathic agents. The essential oils are also important for therapy, their main pharmacological properties being: antimicrobial, antiviral, antifungal, insecticidal, repellent, anti-protozoal,

antioxidant, anti-inflammatory, antimutagenic, eupeptic, carminative, choleretic and vulnerary. One of the mainstream alternative and complementary therapies at the present day is aromatherapy, which uses essential oils as the main therapeutical agents to treat several illnesses. Various applications can also be found in the agricultural, cosmetic and food industries [2,6,9,13,14,23,25,27,29,48].

Objectives

During the period 1971–2014, several researches on plants containing essential oils have been performed by the first author and various collaborators at the department of Pharmaceutical Botany of the Faculty of Pharmacy of „Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, their results being published in different journals. Some of them were continued in PhD studies or for practical applications. The present article aims to present a synthesis of these researches, some of them representing original studies on traditional Romanian medicinal plants, emphasizing their chemical composition and biological action. The species described are: *Tanacetum balsamita* L., *Artemisia abrotanum* L., *A.annua* L., *Inula helenium* L., *Achillea* spp. and *Solidago* spp. (Asteraceae family), *Origanum vulgare* L. and *Salvia officinalis* L. (Lamiaceae), *Rhododendron myrtifolium* Schott et Kotschy (Ericaceae), *Acorus calamus* L. (Araceae), and *Thuja occidentalis* L. (Cupressaceae). The researches concerning each species are presented chronologically.

***Tanacetum balsamita* L.** syn. ***Chrysanthemum balsamita* (L.) Baill.** (**Asteraceae**)

The species is known as alecost, bible leaf, camphor plant, costmary, mint geranium, Patagonian mint, or women's leaf [76]. It has two subspecies: *Tanacetum balsamita* L. var. *tanacetoides* Boiss. (syn. *C. balsamita* var. *tanacetoides* Boiss.), having yellow button-shaped inflorescences (capitula) formed of yellow tubular florets, and *T. balsamita* L. var. *balsamitoides* P.D. Sell (syn. *C. balsamita* var. *balsamita*) that has daisy-like white inflorescences with yellow centres, formed by peripheral white ligulate florets and central yellow tubular florets. Some authors even consider the latter to be a distinct species, *Tanacetum balsamitoides* Sch. Bip. [53,63,76]. Both are cultivated in Romanian gardens for ornamental and aromatic purposes, more often the first variety [63].

The researches performed at The Faculty of Pharmacy in Cluj-Napoca concentrated on the essential oil, as well as on hydro-alcoholic extracts from the two varieties.

The yield of essential oil extracted from the aerial parts during the flowering period was 0.6–1.3% (ml/100g dried plant material) [47]. The main constituent of essential oils extracted from both varieties was identified by gas chromatography: carvone (55–60 %) in the first and camphor (85–90 %) in the second. These results demonstrated that these two morphological varieties correspond to two chemical infraspecific taxa: *C. balsamita* var. *tanacetoides* chvar. *carvone* and *C. balsamita* var. *balsamita,* chvar. *camphora* [60]. Correlated research established the number of chromosomes in the meristem obtained from young roots formed on rhizomes during the spring. This number was 2n=54 (hexaploid) for *C. balsamita* var. *tanacetoides* and 2n=18 (diploid) for *C. balsamita* var. *balsamita* [57].

The two varieties were cultivated at Bod by SC Nivea Braşov, the essential oil and the hydro-alcoholic extracts being used in Tonorelaxin medicinal and cosmetic products [33,34]. The essential oil demonstrated antimicrobial, antifungal and muscle relaxing action [34,56,63]. In rats,

the hydroalcoholic extract demonstrated hepatoprotective action against intoxication with carbon tetracloride and ethanol [50]. The methods for obtaining the essential oil and the hydro-alcoholic extracts were subject to official patents [33,69]. The two varieties were the subjects of two PhD theses [26,32].

***Artemisia abrotanum* L. (Asteraceae)**

Commonly known as southernwood [78], this species originates from southern Europe and is frequently cultivated as an aromatic and ornamental plant [12]. For culture purposes, the plant can be propagated by stem cuttings that easily form roots [51].

The above-mentioned studies led to the quantification of the essential oil from the leaves of the plant: 0.51 % (ml/100 g) in fresh plant material and 2.1% (ml/100 g) in dried [46]. The main constituent of the essential oil was also identified as eucalyptol (1,8-cineole) [46], confirmed by further researches [73]. The essential oil was enriched in eucalyptol through fractioned distillation, its content rising from 29.6% to 76.7%, this resource being considered the richest in eucalyptol in the Romanian flora [5]. The studies concerning the biological activity of the essential oil identified its antimicrobial and antifungal properties [56].

The species was analysed in PhD studies concerning essential oil-containing plants [4].

***Rhododendron myrtifolium* Schott et Kotschy** syn. ***Rh. kotschy* Simonk. (Ericaceae)**

This rhododendron is a small shrub that occurs in the South and East Carpathians, extending to the southern part of the Balkan Peninsula (Bulgarian Rhodope). The plant only grows at higher altitudes and the area of occupancy is less than 500 km^2. At present the species is highly fragmented, several subpopulations being lost, among the main threats being cited grazing and climate change. The species is listed as Endangered globally [12,72].

Tamas and Ciupe (1974) identified brown peltate glands, structures where the essential oil was located, on the lower epidermis of the leaves, using Sudan III reagent (by the red-orange colouration) [58]. The same authors identified a 0.75% (ml/100 g) yield of essential oils in the dried leaves [58]. Further studies performed by thin layer chromatography (TLC) and gas-chromatography (GC) identified as main constituents of the essential oil α and β-pinen, borneol, linalool, and through gas-chromatography and mass selective detection (GC-MSD) were quantified the α-pinen (42.42%), β-selinen (27.66%), β-pinen (5.13%) (21,22). *In vitro* tests concerning the biological action of the essential oil showed reduced antimicrobial and antifungal activity [56]. The studies previously mentioned represent the first researches on the indigenous species *Rh. myrtifolium*.

***Origanum vulgare* L. (Lamiaceae)**

Oregano is a widespread plant species which is native to the Mediterranean, Euro-Siberian and Irano-Turanian regions. It is a well-known medicinal plant indicated to relieve respiratory pathologies such as convulsive cough and colds, as well as skin diseases or digestive disorders. It has also been used from ancient times to flavour traditional dishes [10,11,31].

Even though the Romanian literature on medicinal plants mentions a high content (70%) of phenols (thymol, carvacrol) in the essential oil, by citing data obtained from other *Origanum* species or different *O. vulgare* varieties from southern Europe [9,10,11,24], the amount of phenols in the essential oil from the indigenous species is very reduced or even absent [68]. These results

were confirmed experimentally by Nurzinska-Wierdak et al. (2012), who similarly indicated that the essential oil of *O. vulgare* in Poland does not contain phenolic derivatives [39].

Other comparative studies (GC-MS) with the essential oil of *Thymus vulgaris*, and that obtained from a commercial sample of ‚Oregano' spice, identified respectively 70% and 64% phenolic compounds in these two samples, whereas the main constituents in the indigenous species were sabinene, β-caryophylene, trans-β-ocimene, germacrene-D and ∂-4-carene [64].

***Artemisia annua L.* (Asteraceae)**

This species is commonly known as annual wormwood, sweet Annie or sweet wormwood [78] and is traditionally used in our country for its insecticidal properties. It contains, besides the essential oil, a sesquiterpene lactone, namely artemisinin, that is proven to have antimalarial properties superior to those of synthetic chloroquine [6,7,9,24,27]. These properties are well known, the species being traditionally used throughout Asia and Africa for the treatment of malaria, which led Chinese researchers to the isolation and characterization of artemisinin, for which they were awarded the Nobel Prize for Medicine or Physiology in 2015 [30,79]. We should note that artemisinin is not volatile, its extraction being performed by various techniques other than steam distillation [6]. Recent studies indicate that the plant seems also to have promising antitumour activities [30].

Researches carried out by Popescu, Tămaş and Tibori (1980), the first on the essential oil of this indigenous species, indicated 1.82-2.28% essential oil in the dried plant material, the identified constituents of this plant being artemisiacetone, β-caryophyllen, linalyl acetate, thujone, camphene, borneol, and β-pinene [43].

Other Romanian studies on the species indicated the evolution of artemisinin during the vegetative period using a high performance liquid chromatography technique coupled with mass-spectrometry (HPLC-MS) [28], and the increase in plant biomass through biotechnology (17).

***Inula helenium* L. (Asteraceae)**

Elecampane, called also horse-heal or elfdock, is a widespread plant species in the sunflower family Asteraceae. In some European pharmacopoeias, the roots (*Inulae radix*) are officially listed as diuretic, diaphoretic, expectorant and antihelminthic. The effect on *Mycobacterium tuberculosis* is also mentioned in the literature [10,11,27,75]. It has been demonstrated that the essential oil and extracts from *Inula helenium* roots are rich in sesquiterpene lactones, mainly alantolactone and isoalantolactone [74]. A specific characteristic of the essential oil from the elecampane root is its semi-solid consistency at room temperature (30°C), as compared to other volatile oils that are in liquid state at this temperature.

In order to avoid possible adulterations of the *Inula helenium* root (with the species *Telekia speciosa* or *Atropa belladonna*) Tămaş et al. have developed a TLC technique for highlighting the presence of alantolactone in the root extracts. Also, they have isolated the essential oil with a yield of 2.82% (62.66). Nan et al. (2010) have isolated alantolactone from roots by specific extraction and then investigated it by TLC and IR spectroscopy. GC-MS analysis of the essential oil revealed the presence of alantolactone (63%) and isoalantolactone (36.2%), these representing 99.2% of the isolated essential oil [36,37].

***Salvia officinalis* L. (Lamiaceae)**

The species is commonly known as sage, garden sage or common sage, being a medicinal, ornamental and aromatic plant known from ancient times for its therapeutic properties ("*Qur morietur homo, qui salvia crescit in horto*") [9,10,11,27]. This species was listed in the Romanian Pharmacopoeia, 8th ed. (1965) [18]. Sage is largely used as a savoury food flavouring, either as dried leaves or essential oil. Leaves of *S. officinalis* are used for the treatment of disorders of the oral cavity, mild dyspepsia (such as heartburn and bloating) and gastro-intestinal atony. Also, *S. officinalis* has been used to treat excessive sweating, age-related cognitive disorders, and throat or skin inflammations. [10,11,13,22,59]. The essential oil possesses carminative, antispasmodic, antiseptic, astringent and antihydrotic properties [1].

Tămaş et al. reported a content of 0.85% essential oil in the vegetal product *Salviae folium.* The volatile oil content has been analyzed by TLC and GC-MS. The GC-MS revealed the presence of α-thujone and β-thujone (34.04%), camphor (26.53%), eucalyptol (13.68%), borneol (7.49%), α-pinene (4.88%), camphene (4.30%) and terpineole (2.34%) [59]. An original product, Salviaclim, was produced and used for the treatment of the climacteric syndrome [70].

***Thuja occidentalis* L. (Cupressaceae)**

This tree from eastern North America is commonly known as Arbor vitae or white cedar, and is grown in Europe as an ornamental. The fresh plant contains essential oil, the main constituent being thujone, a toxic compound [3,38]. The tincture of young branchlets and the essential oil of leaves are used in pharmaceutical products for the treatment of warts and papillomas [24,27], for example *Verucolisin* (the tincture obtained from fresh material) [8,54] and *Herbaclear* (the essential oil) [77].

The studies performed at UMF Cluj-Napoca measured the monthly variation in the yield of the essential oil of the branchlets (*Thuja sumitates*), a maximum being found in dried material during August (0.90 ml/100 g). The identification of the components of the essential oil was realized by TLC and GC-MS, and revealed the existence of 23 components; the most important were thujone (53.73%), isothujone (13.47%), fenchone (9.31%) and sequiterpene compounds (8.72%) [61].

***Acorus calamus* L. (Acoraceae)**

The sweet flag or calamus is a semi-aquatic herb growing in shallow water or in a very moist loamy soil. In Romania, two local variants of *A. calamus*, 'De Bega' (from 1980) and 'Ursula' (from 2003) are cultivated for medicinal purposes [35]. The culture of the species was also initiated in Mureș County. Its rhizome contains essential oils, saponins and flavonoids [49]. The plant exhibits polyploidy, and, depending on the β-asarone content of the essential oil, several chemical varieties correlated with the degree of ploidy are known [65]. The rhizome powder was used as a bitter tonic to cure anorexia, dyspepsia and duodenal ulcer (*Ulcerotrat*) [52]. The essential oil has been used in alcoholic beverages, as a fragrant essence in perfumes and oils, and for insecticidal properties [45]. Due to the toxicity (carcinogen) of β-asarone, this main component of the essential oil is limited to a concentration up to 0.01% [55].

The first studies on *A. calamus* from Romania were performed on the commercial product (*Calami radix* from Plafar) and indicated a content of 2.29% essential oil (ml/100g). TLC densitometric determination and UV-spectrometry reported that β-asarone is the major constituent

of both the essential oil and hydro-alcoholic extract. GC-MS analysis of essential oil samples resulted in the identification and quantification of 39 constituents, among which β-asarone represents 10.95%, and this value corresponds to the triploid varieties grown in Europe. In-depth research was carried out by Oprean et al. (1998) to identify the isomers and minor components [40-42].

***Achillea* sp. (Asteraceae)**

The genus *Achillea* has a complex taxonomy. Studies on the composition of the essential oils of *Achillea* spp. have been used as an additional characteristic of inter- and infraspecific differentiation. *Achillea millefolium* L., known as milfoil or common yarrow is considered to be one of the oldest medicinal plants, the vegetal medicinal products used being *Millefolii herba* (aerial part) and *Millefolii flos* (inflorescences) [71]. The Romanian Pharmacopoeia, 10th ed., lists the officinal product *Millefolii flos* with an essential oil content of minimum 0.2% and the Romanian Pharmacopoeia 8th ed. designates an azulene content of at least 12% [18,19].

The studies performed by Tămaş and Popovici investigated the essential oils from the officinal species, as well as the essential oils from the species *Achillea stricta* Gremli (syn. *A. distans* Waldst. & Kit. ex Willd. subsp. *stricta* (Gremli) Janch.), *A. nobilis* L. subsp. *neilrichii* (A. Kern.) Velen, and those from two subspecies of *Achillea distans*: *A. distans* subsp. *distans* and *A. distans* subsp. *alpina* (Rochel.) Soó., harvested from the Rodna mountains in the Eastern Carpathians. *A. distans* subsp. *distans* has white ray florets and *A. distans* subsp. *alpina* (Rochel) Soó has rose-pink ray florets [67]. The aim of these studies was to determine the essential oil content in the inflorescences of the four species, and the azulene content in the isolated essential oil. The essential oil of the inflorescences yielded 0.40% in *A. millefolium*, 0.24% in *A. stricta,* 0.27% in *A. nobilis* subsp. *neilrichii*, 0.40% in *A. distans* subsp. *distans* and 0.25% in *A. distans* subsp. *alpina*. The content of azulenes in the essential oil was 25.26% for *A. millefolium* and 2.41% for *A. stricta*, while in the other species the azulenes are not present [44]. Therefore, the species *A. nobilis, A. distans* and *A. stricta* do not correspond to the standards of the Romanian Pharmacopoeia and they cannot substitute for the officinal species *A. millefolium*.

By GC-MS comparative analysis of essential oils from the two subspecies of *A. distans,* it was observed that two infraspecific taxa (chemotypes) with different chemical composition can be distinguished. In the essential oil of *A. distans* subsp. *distans,* the main constituents were α-thujone (33.31%) and β-thujone (25.52%). In the essential oil of *A. distans* subsp. *alpina*, the thujone is absent and the main constituents were eucalyptol (20.19%), sabinene (6.37%) and camphor (4.94%) [67].

***Solidago* spp. (Asteraceae)**

Three species of the genus *Solidago* have been studied: *Solidago virgaurea* L. subsp. *virgaurea, S. canadensis* L. and *S. gigantea* subsp. *serotina* (Kunze) McNeill (syn. *S. serrotina* Ait.). The herbal product of *S. virgaurea* (*Virgaureae herba*) has been traditionally used to treat the urinary tract, nephrolithiasis and the prostate [9,10,11,24].

S. virgaurea subsp. *virgaurea*, goldenrod, is an indigenous perennial herb with a vertical, cylindrical knotted rhizome, usually without stolons. The yellow flowers are grouped in capitula 10–15 mm across, arranged in long racemes. The ligules of the ray florets are 4–9 mm width. *S. canadensis* L., commonly named Canadian goldenrod, is a rhizomatous, upright herbaceous

perennial plant with numerous stolons and with capitula forming compact erect panicles. The ligules of the ray florets are at most 4 mm in width. *S. gigantea* subsp. *serotina* (Kunze) McNeill (syn. *S. serrotina* Ait.), tall goldenrod or giant goldenrod, is a herbaceous perennial with an erect rhizome and an inflorescence arranged in smaller, compact and pendent panicles. Also, the ligules of the ray florets are at most 4 mm in width. The last two species of *Solidago* are decorative, ornamental plants, native to North America that have become adventive or sub-spontaneous plants in Romania and elsewhere [15].

We found that the essential oil yielded respectively 0.40% in *S. virgaurea,* 0.96% in *S. canadensis* and 0.16% in *S. gigantea.* The chemical composition of the essential oil and the proportion of common compounds are distinct for the three species. Thus, the major compounds for *S. virgaurea* are myrcene (46.30%), α-pinene (13.27%), α-phellandrene (12.57%) and p-cymene (9.27%); for *S. canadensis* they are α-pinene (37.80 %), limonene (24.80 %), germacrene-D (14.08%) and myrcene (8.74%); and for *S. gigantea* they are aristolone (36.73%), germacrene-D (7.5%), sathulenol (6.44%), bornyl acetate (6.04%) and gurjunene (5.22%) [15,16]. In practice, the essential oil of *S. canadensis* is important due this high content of germacrene-D, valued for adding aromatic flavour to same alcoholic beverages.

Conclusions

1. This review summarizes the original researches on essential oils from medicinal, aromatic and ornamental plants of the Romanian flora.

2. By TLC and GC-MS methods the essential oils from seventeen species and varieties, belonging to eleven genera, were qualitatively and quantitatively analyzed.

3. Based on the composition of the essential oils, two infraspecific taxa in *Tanacetum balsamita* and *Achillea distans* were identified.

4. The content of the essential oils and their chemical composition were specified for the first time for *Artemisia abrotanum* and *Rhododendron myrtifolium*.

5. In some of the essential oils analyzed, the content of toxic compounds (β-asarone, thujone) was determined.

6. Contrary to previous data reported up until now, it was specified that the essential oil of *Origanum vulgare* from the Romanian flora does not contain thymol.

7. Some essential oils were tested for antimicrobial and antifungal effects. Also, the possible use of these essential oils in medicinal and cosmetic products was evaluated.

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CERCETĂRI ASUPRA UNOR PLANTE CU ULEIURI VOLATILE EFECTUATE LA UNIVERSITATEA DE MEDICINĂ ŞI FARMACIE „IULIU HAŢIEGANU" CLUJ-NAPOCA

(Rezumat)

Articolul prezintă o sinteză a cercetărilor originale, efectuate la catedra de Botanică farmaceutică a Facultăţii de Farmacie, Universitatea de Medicină şi Farmacie „Iuliu Haţieganu" Cluj-Napoca, asupra a 17 specii și varietăți de plante cu uleiuri volatile, încadrate în 11 genuri, toate fiind specii tradiționale românești, medicinale şi ornamentale, unele dintre acestea fiind endemice. Au fost determinate cantitativ și analizate calitativ, prin CSS și GC-MS, uleiurile volatile izolate din următoarele specii: *Tanacetum balsamita* (2 varietăți), *Artemisia abrotanum*, *Rhododendron myrtifolium, Origanum vulgare, Artemisia annua, Inula helenium*, *Salvia officinalis*, *Thuja occidentalis, Acorus calamus, Achillea* sp. (4 specii) și *Solidago* sp. (3 specii). În urma acestor cercetări s-a precizat compoziția calitativă a uleiurilor volatile din plantele provenite din flora României, au fost identificați 2 taxoni chimici infraspecifici pentru *Tanacetum balsamita* și *Achillea distans* şi s-a precizat conținutul în compuși considerați toxici din uleiurile volatile (β-asarona, thujona).

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