Pharmacogn Rev. 2021;15(30):191-198
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
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Review Article

Valorisation of *Teucrium montanum* as a Source of Valuable Natural Compounds: Bioactive Content, Antimicrobial and Biological Activity – A Review

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ABSTRACT
One of the most popular species among *Teucrium* genus, *T. montanum* has been used as medical herb for generations, but first scientific investigations regarding its bioactive content date back only a few decades ago. Today is known that *T. montanum* is rich in flavonoids and phenolic acids, secondary metabolites with high antioxidant activity and yields relatively high content of essential oil rich in sesquiterpenoids. Most of the published papers investigating *T. montanum* are focused on the characterization of its essential oil that is summarised in the present review paper together with less investigated phenolic content and biological activity resulting from the presence of bioactive compounds.
Key words: Bioactive compounds, Biological activity, Medical herb, Phenolic compounds, *Teucrium montanum.*

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History

- Submission Date: 07-07-2021;
- Review completed: 26-08-2021;
- Accepted Date: 15-09-2021

DOI : 10.5530/phrev.2021.15.18

Article Available online
http://www.phcogrev.com/v15/i30

INTRODUCTION

People looked for medicines in nature since ancient times. Evidence of the use of medical herbs dates back even 5000 years ago in the time of Sumerians and in the time of Chinese Emperor Shen Nung approximately 2500 BC.[1] Dioscorides, "the father of pharmacognosy", is considered as the first writer in history who approached medical botany as applied science and wrote around 80 AD De Materia Medica describing over 600 plant medicines.[2] Willow, chamomile, garlic, onion, marshmallow, ivy, nettle, sage, common centaury, coriander, parsley, sea onion and false hellebore were the most appreciated plants to Dioscorides. Near the same time, Pliny the Elder wrote Historia naturalis about approximately 1000 medicinal herbs.[1] Application of medical herbs was continued through the history from Galen (131 AD – 200), who made the first list of parallel drugs, to Slavic people in the 7th century who used herbs against injurious insects and across the Middle Ages when medical herbs were cultivated in the monasteries and used for therapies and the preparation of drugs. Many medical herbs were brought into Europe after Marco Polo's journeys across Asia, China and Persia (1254 – 1324) and after the discovery of America (1492), as well as after Vasco de Gama's journeys to India (1498). However, the beginning of scientific pharmacy begun much later in the early 19th century with the discovery and isolation of alkaloids from many plants including poppy, ipecacuanha, strychnos, quinine, pomegranate followed by the isolation of glycosides. In the following century, methods for stabilization of medical herbs were evaluated, as well as enhancement of manufacturing and cultivation conditions.[1]

Today, even with developed and widely used modern medicine, medical herbs retained their popularity primarily for historical and cultural reasons, as well because being locally available and cheap. Herbal medicines have become commercially more available and, in some countries, it is a practice to subject them to the same criteria for efficacy, safety and quality as other drug products.[3] According to the WHO,[4] herbal medicines include herbs, herbal materials and preparations, as well as finished herbal products whose active ingredients are part of plants or other plant materials or their combinations. It is estimated that approximately 80% of the global population still relies on the use of herbal medicine as primary health care and natural products and/or natural product structures still have a significant role in the drug discovery and development process.[5,6] Traditional herbal medicine even played a significant role in the strategy for repression and treatment of SARS in China,[7] and similar is assumed for COVID-19.[8] Herbal products are also gaining popularity in Europe where is expected an increase of herbal and traditional products' retail sales from 7.4 billion US dollars in 2010 to 8.8 billion US dollars in 2020.[9] A survey done across the British population about the effectiveness of herbal medicine at treating illness has shown that 44% is of opinion that herbal medicine

Cite this article: Šeremet D, Cebin AV, Mandura A, Komes D. Valorisation of *Teucrium montanum* as a Source of Valuable Natural Compounds: Bioactive Content, Antimicrobial and Biological Activity – A Review. Pharmacog Rev. 2021;15(30):191-8.

is possibly an effective treatment, while 7% considers it as a definitely effective treatment.[10]

Plants of *Teucrium* genus have been used as medical herbs for more than 2000 years.[11] Even today, they are known for diverse biological activities and positive impact on health, resulting from the presence of different secondary metabolites, which is a reason for their usage mainly in pharmacy and ethnobotany medicine.[12] In addition, they can be used in the food industry as spices and in the production of bitter beverages,[13] as well as in the production of flavoured wines and beers.[14] Genus *Teucrium* belongs to *Lamiaceae* family, comprising more than 300 species, mostly perennial herbs, sub shrubs or shrubs. The genus is distributed mainly in the Mediterranean region.[14-16] Regarding common morphological features, leaves of *Teucrium* species are entirely dentate or deeply dissected, flowers are positioned in axils of upper leaves or boom in racemes, panicles or heads, while calyx is often gibbous at base, regular or bilabiate, with five equal or unequal teeth.[17] Aerial parts of *Teucrium* species are covered by an indumentum of glandular and non-glandular trichomes[18] and their micromorphology has been well investigated.[19,20] *Teucrium montanum* L., known as mountain germander, belongs to the section *Polium*, blooms from May to the end of summer and it can be found characteristically in thermophilic limestone and serpentine rocks, dry mountain meadows in Anatoly and Europe.[11] Anatomical and micromorphological studies of *T. montanum* has been carried out and explained by Dinc *et al*.[16] *T. montanum* has been used for generations as analgesic, stomachic, diuretic and antispasmodic agent as well as for its antifungal, antibacterial and antioxidative properties,[21] but first scientific reports about *T. montanum* dates back a few decades ago when a group of Bulgarian scientists was investigating the structure and stereochemistry of newly isolated furanoid diterpenes of clerodane and neo-clerodane types from *T. montanum*. They reported the isolation and structure of montanin-A and montanin-B,[22] montanin-C,[23] montanin-D,[24] montanin-E and montanin-F[25] and montanin-H.[26] Genus *Teucrium* is the most abundant natural source of furanoid diterpenes so *Teucrium* species are accepted as chemotaxonomic markers for neo-clerodanes.[27]

Bioactive content of *Teucrium montanum*

Like all plants, medical herbs synthesize various secondary metabolites, belonging to large chemical classes of alkaloids, terpenoids and phenolic compounds, as a natural defence mechanisms against pathogens and herbivores, as well as for attracting pollinators.[28] Native growing conditions, especially the extreme climate (dry air, direct sunshine and exposition to ultraviolet B radiation, drought stress, etc.), additionally simulate the synthesis of secondary metabolites in plants.[29] Dietary intake of some of these compounds by plant or plant-based food consumption can substantially lead to various health benefits. Biological roles and future perspectives of natural alkaloids as drugs have been discussed by Qiu *et al*.,[30] antioxidant properties and beneficial health effects of phenolics and polyphenolics by Shahidi and Ambigaipalan[31] and for terpenoids by de las Heras *et al*.[32] Physiological activities of *Teucrium* species are mainly attributed to the presence of phenolic compounds and terpenoids.[33]

Phenolic content and antioxidant capacity of *Teucrium montanum*

Phenolic compounds are ubiquitous in plants where they can be found in a variety of chemical structures, ranging from a simple phenolic molecule to complex high molecular weight polymers. From a physiological aspect, phenolic compounds are known as free radical scavengers with strong antioxidant activity resulted from the presence, arrangement and number of hydroxyl groups.[31] Collected data from the available literature regarding total phenolic, flavonoid and phenolic acid content of *T. montanum*, together with applied extraction parameters, are summarized in Table 1, while summary regarding the content of individual phenolic compounds is presented in Table 2.

Among the first, in 1986, Harborne *et al*.[34] published paper describing flavonoid content in 42 European taxa of the *Teucrium*, including also *T. montanum* in which the presence of cirsiliol, cirsimaritin, cirsilineol, luteolin, cirsimaritin, vicenin-2, cynaroside, isoquercitrin and rutin (Table 2) were detected using chromatographic analyses on silica gel TLC and two-dimensional chromatography. In the following years, phenolic content of *T. montanum* was further investigated, but more advanced analytical methodology was used. Tumbas *et al*.[21] extracted phenolics from *T. montanum* using different solvents (methanol, petroleum ether, chloroform, ethyl acetate, 1-butanol and water) and then the content of individual phenolic compounds using high

Table 1: Total phenolic, flavonoid and phenolic acid content in *T. montanum* with applied extraction parameters.

Country of origin	Extraction parameters	Total phenolic content	Flavonoid content	Phenolic acids content	Ref.
Region of Zlatibor, Serbia	20 g of plant material, 2x500 mL of 70% methanol, room temperature, 2×24 h, successively treatment with petroleum ether (2×20 mL), chloroform (2×20 mL), ethyl acetate (2×20 mL) and 1-butanol (2×20 mL)	296 mg CAE/g (1-butanol extract)	/	28.62 mg/g (ethyl acetate extract)	[21]
Region of Goc Mt., Serbia	10 g of plant material, extraction with organic solvent (water, methanol, acetone, ethyl acetate, petroleum ether), volume of solvent used unknown	169 mg GAE/g extract (methanol extract of the whole plant)	88.31 mg RUE/g extract (acetone extract of leaves)	/	[12]
Serbia	10 g of plant material, 250 mL of methanol, room temperature, 24 h	190.20 mg GAE/g extract	54.19 mg RUE/g extract	/	[40]
Serbia	Subcritical water extraction, sample/solvent ratio 1:10, 160 °C, 30 min, 10 bar	174.61 mg GAE/g DE	/	/	[42]
Balkan Peninsula, Serbia	Microwave-assisted extraction, 5 g of plant material, 150 mL of 50% ethanol, 30 min, 450 W	73.60 mg GAE/g DE	65.31 mg RUE/g DE	/	[29]

DE=dry extract; CAE – Chlorogenic acid equivalent; GAE – Gallic acid equivalent; RUE – Rutin equivalent

Table 2: Content of individual phenolic compounds in *T. montanum*.

Hydroxyl derivatives of benzoic acid

Gallic acid	*Gentisic acid*	*Protocatechuic acid*	*Vanillic acid*	*Syringic acid*	*Ref.*
0.132 mg/g DW[a]	14.432 mg/g DW[a]	1.337 mg/ g DW[a]	1.944 mg/g DW[a]	4.588 mg/g DW[a]	[21]
3.45 mg/g DE[c]	n.d.	1.17 mg/g DE[c]	0.453 mg/g DE[c]	n.d.	[42]

Hydroxyl derivatives of cinnamic acid

Chlorogenic acid	*Neochlorogenic acid*	*Caffeic acid*	*p-coumaric acid*	*Ferulic acid*	*Rosmarinic acid*	*3,5-dimethoxy-4-hydroxycinnamic acid*	*Ref.*
3.076 mg/g DW[a]	n.d.	0.515 mg/g DW[a]	1.794 mg/g DW[a]	0.811 mg/g DW[a]	n.d.	0.252 mg/g DW[b]	[21]
0.799 mg/g DE[c]	n.d.	0.560 mg/g DE[c]	n.d.	n.d.	n.d.	n.d	[42]
n.d.	0.2 mg/g DW[d]	0.04 mg/g DW[d]	n.d.	n.d.	n.d.	n.d	[36]
n.d.	n.d.	n.d.	n.d.	n.d.	+	n.d	[35]

Flavonoid aglycones and glycosides

(+)-catechin	*Epicatechin*	*Naringin*	*Rutin*	*Luteolin*	*Luteolin-7-O-rutinoside*	*Luteolin-7-O-glucoside*	*Apigenin*	*Quercetin-3-O-rutinoside*	*Ref.*
1.15 mg/g DE[c]	1.20 mg/g DE[c]	9.96 mg/g DE[c]	1.25 mg/g DE[c]	n.d	n.d	n.d	n.d	n.d	[42]
n.d	n.d	n.d	+	+	n.d	n.d	n.d	n.d	[34]
n.d	n.d	n.d	n.d	0.1 mg/g DW[d]	0.6 mg/g DW[d]	0.1 mg/g DW[d]	0.04 mg/g DW[d]	1.0 mg/g DW[d]	[36]
n.d	n.d	n.d	n.d	+	n.d	n.d	n.d	n.d	[35]
Diosmetin	*Diosmetin-7-O-rutinoside*	*Isoquercitrin*	*Vicenin-2*	*Cirsiliol*	*Cirsilineol*	*Cirsimaritin*	*Cynaroside*	*Chrysoeriol*	*Ref.*
+	n.d	n.d	n.d	n.d	n.d	n.d	n.d	+	[35]
n.d	0.4 mg/g DW[d]	n.d	n.d	0.7 mg/g DW[d]	n.d	0.5 mg/g DW[d]	n.d	n.d	[36]
n.d	n.d	+	+	+	+	+	+	n.d	[34]

Phenylethanoid glycosides

Caerulescenoside	*Castanoside A*	*Echinacoside*	*Forsythoside B*	*Verbascoside*	*Samioside*	*Ref.*
7.0 mg/g DW[d]	1.1 mg/g DW[d]	2.4 mg/g DW[d]	10.2 mg/g DW[d]	2.0 mg/g DW[d]	1.7 mg/g DW[d]	[36]

DW= dry weight; DE=dry extractn.d.=not detected; +=identified, but not quantified; a=determined in ethyl acetate extract; b=determined in 1-butanol extract; c=subcritical water extraction; d=determined in methanolic extract

performance liquid chromatography (HPLC) coupled with diode-array detector (DAD) was determined. Total phenolic content ranged from 32.40 mg CAE/g in ethyl acetate extract to 296 mg CAE/g in 1-butanol extract (Table 1), while in petroleum ether extract no phenolics were observed. The highest content of phenolic acids (28.62 mg/g) was observed in ethyl acetate extract with gentisic acid (14.43 mg/g) as a major compound. Among other phenolic acids, gallic, protocatechuic, vanillic and syringic acid were identified from the group of hydroxyl derivatives of benzoic acid, while from the group of cinnamic acid hydroxyl derivates, chlorogenic, caffeic, *p*-coumaric, ferulic and 3,5-dimethoxy-4-hydroxycinnamic were identified (Table 2). Panovska *et al.*[35] reported the presence of rosmarinic acid, luteolin, chrysoeriol and diosmetin in *T. montanum*, also using HPLC-DAD methodology, as well as a significant inhibitory effect of diethyl ether (45%), ethyl acetate (45%) and *n*-butanol extracts (46%) of *T. montanum* on the production of hydroxyl radical assessed by the iron(II)–dependent deoxyribose damage assay. In addition, the diethyl ether extract showed the highest inhibitory activity against DPPH radical achieving 50% of its inhibition at a concentration of 10 mg/mL. Mitrevski *et al.*[36] used more advanced chromatographic analysis - LC/DAD/ESI-MS^n, and reported that neochlorogenic acid can also be found in *T. montanum*. The same authors[36] also reported about presence of phenylethanoid glycosides - glycosides consisting of phenylethyl alcohol and glycosyl moieties,[37] in *T. montanum* such as caerulescenoside, castanoside A, echinacoside, forsythoside B, verbascoside and samioside, as well as about different glycosides present in both aglycone and glycone forms (Table 2). Further, Stankovic *et al.*[12] studied the extraction of phenolic compounds from different parts of *T. montanum*, including flowers, leaves and steams, separately, and the whole plant, using water, methanol, acetone, ethyl acetate and petroleum ether as solvents. Methanol extracts of the whole plant and water extract of leaves exhibited the highest total phenolic content of 169 mg GAE/g extract and 154.81 mg GAE/g extract, respectively. Flavonoid content ranged between 3.96 mg RUE/g extract in petroleum ether extract of the whole plant to 88.31 mg RUE/g extract in the acetone extract of leaves (Table 1). However, water extracts showed the highest capacity to neutralize DPPH radicals, especially the water extract of the whole plant, resulting in 50% inhibition at a concentration of 29.41 μg/mL (IC_{50}). In the study of Djilas *et al.*[38] ethyl acetate and *n*-butanol extracts of the aerial parts of *T. montanum* showed a significant free radical scavenging activity by removing 58.79

and 100%, respectively, of DPPH free radicals determined by electron spin resonance. Further, Čanadanović-Brunet *et al*.[39] studied the ability of *T. montanum* extracts to scavenge reactive hydroxyl radicals, during the Fenton reaction, using electron spin resonance spectroscopy, and lipid peroxyl radicals obtained during lipid peroxidation. Among investigated solvents (methanol, petroleum ether, chloroform, ethyl acetate and water), *n*-butanol extract exhibited the highest antioxidant activity eliminating 100% of hydroxyl radicals when present at a concentration of 0.16 mg/mL, 100% of peroxyl radicals formed during AAPH-induced lipid peroxidation at concentration of 5 mg/mL and 90.57% of the same radicals formed during ACVA-induced peroxidation of sunflower oil at equal concentration. Zlatić *et al*.[40] reported higher content of total phenolic compounds and flavonoids for *T. montanum* methanolic extracts sampled from serpentine localities (160.21 – 190.20 mg GAE/g extract and 53.82 – 54.19 mg RUE/g extract, respectively) than for samples from calcareous localities (143.42 – 148.21 mg GAE/g extract and 46.50 – 49.53 mg RUE/g extract, respectively). According to Jurišić Grubešić *et al*.,[41] native *Teucrium* species are richer in total phenolic content than cultivated ones and, among all investigated species, *T. montanum* was found to contain the highest content of phenolics, accounting for 13.68% DW.

Unlike the aforementioned papers where phenolic compounds were mostly extracted using conventionally techniques based on the mixing of the plant material with different organic solvent, Nastić *et al*.[42] and Vujanović *et al*.[29] for the same purpose applied subcritical water extraction and microwave-assisted extraction, respectively. Nastić *et al*.[42] studied the impact of temperature and pressure in the subcritical water extraction technique on the yield of total phenolics and antioxidant characteristics of the extracts. The highest values (174.61 mg GAE/g DE (TPC), 176.23 mg TE/g DE (DPPH) and 141.71 mg AAE/g DE (FRAP)) were obtained by combining temperature of 160 °C and pressure of 10 bar. HPLC-DAD analysis showed naringin (996 mg/100 g DE) and gallic acid (345 mg/100 g DE) as predominant phenolic compounds, as well as the presence of protocatechuic, chlorogenic, vanillic, caffeic and ferulic acids and flavonoids (+)-catechin, epicatechin and rutin (Table 2). Vujanović *et al*.[29] studied the application of microwave-assisted extraction and obtained the highest total phenolic (73.60 mg GAE/g DE) and flavonoid contents (65.31 mg RUE/g DE) for 30 min, 450 W extraction using 50% ethanol as solvent (Table 1). The antioxidant activity of the extract, evaluated by ABTS, CUPRAC, phosphomolybdenum and metal chelating assays, showed high scavenging activity and reducing power and indicated high efficiency towards reaction based on single electron transfer (74.29 mg TE/g (ABTS), 125.91 mg TE/g (CUPRAC), 1.09 mmol TE/g (phosphomolybdenum) and 1.38 mg EDTAE/g (metal chelating)).

Data collected from the available literature confirmed *T. montanum* as a significant source of phenolic compounds with high antioxidant activity, thus indicating its potential usage in the pharmacology and food industry for the production of functional foods and food supplements.

Essential oil and terpenoid content of *Teucrium montanum*

The essential oil producing species are widespread across the plant kingdom. Essential oil of plants is usually obtained by steam distillation of leaves, plant reproductive parts, stem or roots. Its composition is very complex, with terpenoids as predominant compounds.[43] Terpenoids can be defined as secondary metabolites of plants derived from the basic branched C5 unit - isoprene and can be grouped into hemi-, mono-, sesqui-, di-, sester-, tri-, and tetraterpenoids containing 1, 2, 3, 4, 5, 6, and 8 isoprenoid residues, respectively.[32]

Glandular trichomes, secretory structures that produce and store essential oils in plants,[44] have been reported among *Teucrium* species.[20,45] Particularly, in the case of *T. montanum*, yield of essential oil ranges between 0.15 and 0.47%.[33,46-51] Essential oils of *Teucrium* species are known to be rich in sesquiterpenoids,[14,18] and similar was reported for *T. montanum*. Radulović *et al*.[51] reported that sesquiterpenoids comprised 72.7% of *T. montanum* essential oil, particularly, 39.3% fell on sesquiterpene hydrocarbons and 33.4% on oxygenated sesquiterpenes, while monoterpenoids accounted for 22% - 7.9% for monoterpene hydrocarbons and 14.1% for oxygenated monoterpenes. Similar was reported by Pavela *et al*.,[49] including the dominance of oxygenated sesquiterpenes (39.7%) followed by sesquiterpene hydrocarbons (36.6%). A slight different composition was reported by Bezić *et al*.[47] who identified 37 different compounds in water distilled essential oil of *T. montanum* comprising sesquiterpenes (35.1%), monoterpenes (28.4%), oxygenated monoterpenes (12.4%), oxygenated sesquiterpenes (5.1%) and carbonylic compounds (0.9%). In the study by Baser *et al*.[48] sesquiterpenes were also predominated over monoterpenes in the essential oil of *T. montanum*, and about 1/3 of the sesquiterpenes consisted of oxygenated sesquiterpenes. In the study of Catinella *et al*.,[50] oxygenated sesquiterpenes made up as much as 63.5% of *T. montanum* essential oil, while sesquiterpene hydrocarbons were represented by 30.8%.

Summarized results of the composition of *T. montanum* essential oil collected from the available literature are presented in Table 3. As can been concluded from the presented results, different individual compounds were found to be dominant according to the origin of the plant. Kovacevic *et al*.[33] reported germacrene D (15.0%), α-pinene (12.4%), β-eudesmol (10.1%) and β-caryophyllene (6.9%) as main constituents of the *T. montanum* essential oil, while Vukovic *et al*.[46] for the *T. montanum* collected in the same county (Serbia) reported the dominance of δ-cadinene (17.19%), β-selinene (8.16%) and α-calacorene (4.97%). Sabinene (11.3%), δ-cadinene (6.3%), germacrene D (5.8%) and α-copaene (5.7%) were determined as the main essemtial oil constituents among approximately 120 compounds in *T. montanum* originated from Turkey (Table 3).[48] Longifolenaldehyde (14.5%), epiglobulol (13.5%) and ledene oxide (12.1%) were found to be the most represented in the *T. montanum* essential oil originated from Slovakia,[49] while β-pinene (12.3%), germacrene D (17.2%) and β-caryophyllene (7.1%) for the *T. montanum* that inhabits Croatia.[47]

Antimicrobial and antiviral activity of *Teucrium montanum*

Antimicrobial activity of plants is generally attributed to the presence of phenolics, terpenoids, essential oils, alkaloids, lectins, polypeptides and polyacetylenes.[52] As presented in previous sections, extracts and essential oils obtained from *T. montanum* contain valuable natural compounds with potential antimicrobial activity.

In the study of Djilas *et al*.[38] ethyl acetate and *n*-butanol extracts of the aerial parts of *T. montanum* showed significant activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*, determined by the disc diffusion technique and agar dilution assay method, while not exhibiting any activity against yeasts (Table 4). Similar results were reported by Stanković *et al*.[13] who studied *in vitro* antimicrobial activity of *Teucrium* species for different Gram-positive and Gram-negative bacterial species and as well as for fungal species, using microdilution method with resazurin. Methanol extract showed the highest inhibitory effects, especially against bacteria species resistant to amoxicillin *S. aureus* ATCC 25923 and *P. aeruginosa*, while antifungal activity was not observed (Table 4). The authors noted a positive correlation between phenolic content and antimicrobial activity. In the study by Vukovic *et al*.,[46] the essential oil of *T. montanum* obtained by hydrodistillation, showed greater antibacterial and antifungal effect than *T. montanum* methanolic extract. In the study of Bezić *et al*.,[47] the essential oil of

Table 3: Composition of *T. montanum* essential oil.

Country of origin	Oil extraction	Oil extraction yield (%)	Main constituents (> 2.5%)	Ref.
Mountain Orjen, Serbia	Air dried aerial parts, hydrodistillation for 3h	0.15	germacrene D (15.0%), α-pinene (12.4%), β-eudesmol (10.1%), β-caryophyllene (6.9%), β-pinene (4.8%), δ-cadinene (4.1%), γ-cadinene (4.5), α-cadinol (3.5%), cadinol (3.6%), bicyclogermacrene (3.5%), caryophyllene oxide (2.6%)	[33]
Mountain Jadovnik, Serbia	Aerial parts, hydrodistillation for 3 h	0.47	δ-cadinene (17.19%), β-selinene (8.16%), , α-calacorene (4.97%), 1,6-dimethyl-4-(1-methylethyl)- naphthalene (4.91%), caryophyllene (4.35%), copaene (4.23%), torreyol (3.91%), 4-terpineol (3.90%), cadina-1,4-diene (3.39%), Sesquiphellandrene (3.34%), γ-curcumene (3.18%), τ-cadinol (3.12%), α-cedrene (2.90%)	[46]
Elevations between Trogir and Prapatnica, Croatia	Aerial parts, hydrodistillation for 3 h	0.4	β-pinene (12.3%), germacrene D (17.2%), β-caryophyllene (7.1%), limonene (4.6%), myrcene (4.2%), linalool (3.6%), β-bourbonene (3.4%), hexacosane (3.4%), pentacosane (3.3%), tetracosane (3.1%), *(Z)*-β-farnesene (2.9%), tricosane (2.8%), δ-cadinene (2.7%), heptacosane (2.7%)	[47]
Sipyl mountain, Turkey	Air dried aerial parts, hydrodistillation for 3h	0.02	sabinene (11.34%), δ-cadinene (6.25%), germacrene D (5.80), α-copaene (5.69%), farnesene (5.53%), *(E)*-β-farnesene (5.53%), τ-cadinol (5.45%), α-pinene (5.16%), linalool (3.25%), β-pinene (3.07%), α-cadinol (2.56%)	[48]
Slovak Karst, Slovakia	Aerial parts, hydrodistillation for 3 h	0.19	germacrene D (12.8%), *(E)*- caryophyllene (8.0%), *epi*-α-cadinol (4.5%), α-pinene (3.1%), bicyclogermacrene (3.1%), *epi*-cubebol (3.0%), cubebol (3.0%), δ-cadinene (2.7%), caryophyllene oxide (2.5%)	[49]
Contrada Quacella on the Madonie Mountains, Sicily, Italy	Aerial parts, hydrodistillation for 3 h	0.07	longifolenaldehyde (14.5%), epiglobulol (13.5%), ledene oxide (12.1%), β-cedrene (8.9%), 8-cedren-13-ol (5.7%), α-funebrene (4.5%), globulol (4.5%), β-bisabolol (3.9%), dehydroaromadendrene (3.0%), caryophyllene oxide (2.8%), cubenol (2.8%), α-humulene (2.5%)	[50]
Jabuka, Serbia	Aerial parts, hydrodistillation for 2.5 h	0.19	δ-cadinene (8.1%), *β*-caryophyllene (5.1%), τ-muurolol (4.2%), α-pinene (4.0%), dehydrosesquicineole (3.9%), γ-cadinene (3.6%), α-cadinol (3.5%), α-humulene (3.1%), *trans*-verbenol (2.9%),	[51]

Table 4: Antibacterial and antifungal activity of *T. montanum* extracts.

Extract	*E. coli* ATCC 25922		*S. aureus* ATCC 25923		*E. coli*		*S. aureus*		*P. aeuruginosa*		*C. albicans* ATCC 10231		*C. albicans*		*A. niger*		*S. lutea*		*Bacillus* sp.		Ref.
	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC	
Methanol	5	5	0.15	0.3	5	5	5	5	2.5	5	>20	>20	>20	>20	>20	>20	/	/	/	/	
Acetone	10	10	0.15	0.15	20	20	10	10	5	>20	>20	>20	>20	>20	20	20	/	/	/	/	**[13]**
Ethyl acetate	>10	>10	0.3	0.3	>10	>10	>10	>10	10	>10	>10	>10	>10	>10	>10	>10	/	/	/	/	
	/	/	/	/	>10	>10	1.0	2.5	2.5	5.0	/	/	/	/	/	/	10	>10	7.5	10	
Petroleum ether	/	/	/	/	>10	>10	>10	>10	>10	>10	/	/	/	/	/	/	10	>10	10	>10	[38]
Chloroform	/	/	/	/	10	>10	5.0	5.0	10	10	/	/	/	/	/	/	10	>10	10	>10	
n-butanol	/	/	/	/	>10	>10	1.0	2.5	2.5	5.0	/	/	/	/	/	/	10	>10	7.5	10	

MIC (minimum inhibitory content) and MMC (minimum microbicidal content) values are expressed in mg/mL

T. montanum showed the strongest antiphytoviral activity of the evaluated *Teucrium* species, by reducing the number of lesions in the local host *Chenopodium quinoa* infected with Cucumber Mosaic Virus for 44.3%, probably due to higher content of germacrene D, β-pinene and limonene.

Summarized results of antimicrobial activity of *T. montanum*, including the values of the minimum inhibitory content (MIC) and minimum microbicidal content (MMC) are presented in Table 4. From the presented studies it can be observed that crude extracts and essential oils of *T. montanum* possess a great potential for medicinal and pharmaceutical applications as antimicrobial and preservative agents.

Biological activity of *Teucrium montanum*

Biological activity of plant extracts by means of protective properties and anti-proliferative power, depends on their content of various phytochemicals that can affect numerous target molecules of signaling pathways in the malignantly transformed cells, both in basic and structurally modified forms, thus exerting antitumor or cancer prevention properties.[29,53]

Additionally, complex mixtures of different compounds, such as plant extracts, can achieve higher efficacy than single-compound based drugs, due to synergistic effects, making them effective in lower dosage while also lowering adverse toxicity issues.[52] It is estimated that approximately 60% of used anticancer chemotherapeutic drugs are derived from natural sources, including plants, marine organisms and micro-organisms.[54]

Vujanović *et al*.[29] analysed the cytotoxic activity of *T. montanum* extract, obtained by microwave-assisted extraction with 50% ethanol, on RD (cell line derived from human rhabdomyosarcoma), Hep2c (cell line derived from human cervix carcinoma — HeLa derivative) and L2OB (cell line derived from murine fibroblast) malignant cell lines by MTT assay. *T. montanum* extract at concentrations of 34.35, 13.45 and 18.37 μg/mL matching IC_{50} values inhibited cell survival of Hep2c, RD and L2OB cell lines, respectively, by 50 %. The authors concluded that *T. montanum* extract was effective only for RD and L2OB cell lines. In addition, the authors reported on the inhibitory effects of *T. montanum* extract against α-amylase and α-glucosidase, the key enzymes in carbohydrate digestion the inhibition of which is an important therapeutic strategy to manage postprandial blood glucose peaks. *T. montanum* extract showed moderate inhibitory activity against enzymes α-amylase and α-glucosidase achieving an inhibitory of 0.58 and 4.55 mmol ACAE/g extract, respectively.[29] On the other hand, the activity against tyrosinase, the main enzyme in the synthesis of melanin, the inhibition of which could be used in the treatment of hyperpigmentation problems, was rather low (4.62 mg KAE/g extract). Milošević-Djordjević *et al*.[11] evaluated genotoxic potential of *T. montanum* methanolic extract on cultured human peripheral blood lymphocytes (PBL) using cytokinesis-block micronucleus (MN) assay. Cultures were treated with the extract of different concentrations (125, 250, 500 and 1000 μg/mL), both separately and in combination with a chemotherapeutic agent mitomycin C (MMC). The results showed that only the highest concentration significantly induced MN frequency in PBL, while only the lowest decreased the mutagenic effects of MMC. The results indicated *T. montanum* methanolic extract as a potential chemoprotective drug in cancer therapy when used in small dosage. Stanković *et al*.[55] studied the antiproliferative activity and antioxidant properties of methanolic extracts from different *Teucrium* species, including *T. montanum*, *in vitro* (HCT-116 human colon cancer cell line). They reported significant inhibition of cell growth for all *Teucrium* extracts, determined by MTT assay, in a dose-dependent manner after 24 and 72 h of treatment. *T. montanum* extract showed a pronounced cytotoxic effect after 24 h of exposure inhibiting the growth of HCT-116 cells by 50% at a concentration of 1.08×10^{-5} μg/mL (IC_{50} value). Additionally, a high correlation between antiproliferative activity and the content of phenols was observed for almost all *Teucrium* extracts. As the authors explained, the observed inhibitory activity could be supported by modifications of redox status and interference with basic cellular functions. *T. montanum* extract showed increased percentages of early apoptotic (54.02%), late apoptotic (37.93%) and increased percentage of necrotic cells (6.32%), compared to the spontaneous apoptosis occurred in control cells.[55] All *Teucrium* extracts exhibited a strong antioxidant activity after 72 h, reducing both, levels of O_2- and NO_2- production. Specifically, *T. montanum* extract showed a remarkable ability to reduce the level of NO_2- after 72 h of exposure. [55]

CONCLUSION

Teucrium montanum, commonly known as mountain germander, presents a rich source of natural bioactive compounds, including phenolic compounds, especially phenolic acids, and terpenoids. Many studies have confirmed the strong antioxidant activity of its extracts, as well as a remarkable antimicrobial and biological activity. Apart from traditional uses, new insights into the chemical and bioactive composition of *T. montanum* contributes to its further popularization and increased valorisation aimed at new, scientifically-based application incorporation into various food products, such as the development of new formulations of functional products.

ACKNOWLEDGEMENT

This paper has been realized under the project "Formulating encapsulated systems of bioactive ingredients from traditional plants: mountain germander and ground ivy for the development of innovative functional food products" (IP-2019-045879), funded by the Croatian Science Foundation.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

ABBREVIATIONS

AAE: Ascorbic acid equivalent; **AAPH:** 2,2'-azobis(2-amidino-propane) dihydrochloride; **ACAE:** Acarbose equivalents; **ACVA:** 4,4'-azobis(4-cyanovaleric acid); **CAE:** Chlorogenic acid equivalent; **CUPRAC:** Cupric ion reducing antioxidant capacity; **DE:** Dry extract; **DW:** Dry weight; **GAE:** Gallic acid equivalent; **KAE:** Kojic acid Equivalent; **RUE:** Rutin equivalent; **TPC:** Total phenolic content; **TE:** Trolox equivalent.

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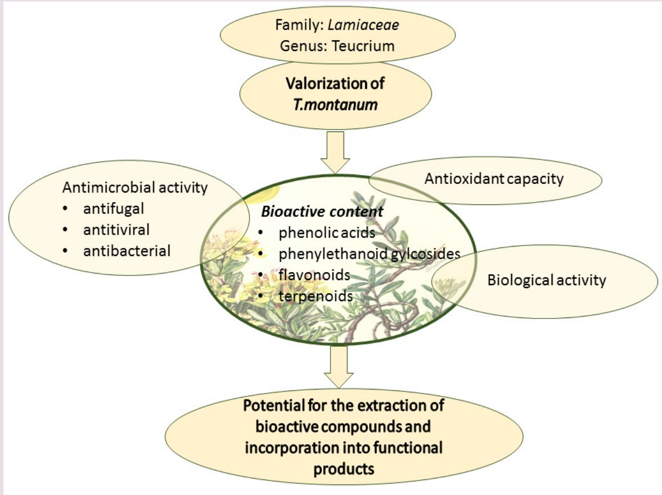
GRAPHICAL ABSTRACT



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Cite this article: Šeremet D, Cebin AV, Mandura A, Komes D. Valorisation of *Teucrium montanum* as a Source of Valuable Natural Compounds: Bioactive Content, Antimicrobial and Biological Activity – A Review. Pharmacog Rev. 2021;15(30):191-8.