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REVIEW ARTICLE

Ethnopharmacology, Chemical Composition and Pharmacological Activity of Amethyst Sea Holly (*Eryngium amethystinum* L)

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Abstract

Eryngium amethystinum L is a plant which grows in Balkan and Apennine peninsulas. The aerial parts, roots and fruits are used in ethnopharmacology of Italy and Western Balkan countries. Traditional preparations of *E amethystinum* were used in treatment of oedema, malaria and gastrointestinal diseases. Essential oils and extracts were isolated from aerial parts and roots of *E amethystinum* and examined by antimicrobial, antioxidative and cytotoxic assays. Gas-chromatography analysis showed predominance of germacrene D and spathulenol in essential oils of *E amethystinum* aerial parts, which have contributed to strong cytotoxic activity, while the methanolic extract exhibited strong antioxidative and antimicrobial activity. This article summarises all existing knowledge regarding *E amethystinum*, its chemical composition and pharmacological activity.

Key words: *Eryngium amethystinum*; Essential oils; Anti-infective agents; Oxidative stress; Cytotoxic activity.

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Introduction

Eryngium amethystinum L is a member of the Apiaceae family. The Apiaceae family is an abundant source of various traditional medicinal plants. Modern medicine confirmed the significance of certain species from this family as a valuable source of pharmacologically active molecules.[1] The Apiaceae family is characterised with presence of ducts and vittae loaded with oil and mucus.[2] Many members of Apiaceae family are used for industrial purposes and for nutrition as well.[3] The plants from Apiaceae family are extensively studied to examine their antibacterial, anti-inflammatory, antioxidative and diuretic activities.

The focus of review is primarily on *E amethystinum* L, which belongs to the "Old World" clade of Eryngium, the most abundant genus within the Apiaceae family.[4] Considering long-standing usage of *E amethystinum* in Bosnia and Herzegovina, primarily as the cure for oedema.[5] It is of great significance to conduct literature research which covers usage in traditional medicine, chemical

composition and pharmacological activity of *E amethystinum*. To the best of our knowledge, this is the first article which summarises existing knowledge regarding these aspects of *E amethystinum.* The aim of this review was to focus on *Eryngium amethystinum* var *amethystinum*, which grows in the central parts of Bosnia and Herzegovina and to gather existing knowledge about this plant and its therapeutic value.

Taxonomy of *Eryngium amethystinum* L

The geographic position of Balkan Peninsula is unique, being on the crossroad between Asia and Europe. Balkan Peninsula is situated between two main centres of diversity within the genus Eryngium. This specific location has influenced diversity of plant properties within species belonging to the genus Eryngium growing in this area. Subgeneric classification recognises five subgenera, with *Eryngium* subgenus *Eryngium* being the most important for the Balkans. All of 11 *Eryngium* species in the Balkan belong to this subgenus (Table 1). The main characteristic of this subgenus is palmately division of basal leaves, although basal leaves can have trifoliate shape or pinnate segments.[6] *E amethystinum* is a perennial plant with an elongated, cylindrical stem. The stem grows over 50 cm; it grows vertically and carries sturdy, spaced leaves and prominent branches. The leaves are firm; the lower leaves have petioles. The shape of lower leaves is oval and bipin-

Figure 1: Eryngium amethystinum L growing in central Bosnia and Herzegovina, Kupres Municipality

Table 1: *Eryngium species present in Balkan peninsula*

Species	Remarks
Eryngium amethystinum	Distributed in Adriatic basin, two varieties
Eryngium amorginum	Endemic to Aegean Sea, the only chasmophyte in genus *Eryngium*
Eryngium campestre	Widespread across Europe
Eryngium glomeratum	Common in Eastern Mediterranean, occasionally found in Crete
Eryngium serbicum	Endemic for central Balkans, especially Serbia
Eryngium ternatum	Endemic to Crete
Eryngium alpinum	Grows on moist terrains, colonisation due to Ice Ages
Eryngium creticum	Widespread across Eastern Mediterranean
Eryngium palmatum	Lacks spikes, endemic to Balkan Peninsula
Eryngium wiegandii	Schlerophyllus habitat, endemic to Balkans
Eryngium maritimum	Located at the sea coasts, endangered by tourism

nate, with lobes at the base of the leaf being less pronounced. Lobes of second rows are narrow or broadly linear, with varying length. The upper leaves have shorter petioles and are palmately divided. The umbels are densely distributed and shield-shaped. The flower heads are oval-shaped, up to 15 mm long, located on thickened dichotomous petioles. The involucral leaves are linear-lanceolate either obtuse or sharp, two to five times longer than heads. The sepal leaves are oval-lanceolate, either obtuse or sharp, around two mm long and are the same length as the petal leaves or slightly longer. Petal leaves are blue, elongate oval or almost quadrangular and deeply cut (Figure 1).[7]

Considering taxonomic subdivision of *Eryngium amethystinum*, this plant has three varieties, specifically distributed throughout Balkan Peninsula. The first variety is *Eryngium amethystinum* var *amethystinum*, located at the northern part of Balkan Peninsula. The second variety is *Eryngium amethystinum* var *tenuifolium*, located at the southern part of Balkan Peninsula, characterised with narrower leaves than *Eryngium amethystinum* var *amethystinum*; this variety is common to hot climate areas. The third variety is *Eryngium amethystinum* var *transiens*, as transient variety of *E amethystinum*, located geographically between two habitat areas.[8]

Ethnopharmacology of *Eryngium amethystinum*

The genus *Eryngium* is an abundant source of plants used in traditional medicine worldwide (Table 2). *E amethystinum* is a plant employed in traditional medicine of Italy, Croatia and Bosnia and Herzegovina. In Italian folk medicine, this plant is used as a diuretic and laxative.[9] *E amethystinum* roots in Italy are also used in the treatment of oedema, acidosis and urinary infections. In the Amiata Mountain area of Italy, the roots of *E amethystinum* are used as a diaphoretic and for the treatment of cellulite.[10] This action on skin can be compared with *E foetidum* L leaves from Ecuador, which are used to cure skin conditions.[9] In Italian traditional medicine in the area of Montecorvino, southern Italy, flowers of *E amethystinum* are used to prevent malaria. In the northern part of Dalmatia, Croatia, young shoots and roots are eaten by locals and young leaves are used in combination with other naturally occurring plants.[11] Aerial parts of *E caucasicum* Trautv from Iran, are also used as additive in the food.[12] In central parts of Bosnia and Herzegovina, *E amethystinum* aerial parts were used to treat "water sickness" (the colloquial name for oedema) and hepatitis as well.[5] Ground parts of *E amethystinum* were used for preparation of salads and beverages during Bosnian War (1992-1995).[13] Ethnopharmacology of *E amethystinum* is summarised in Figure 2.

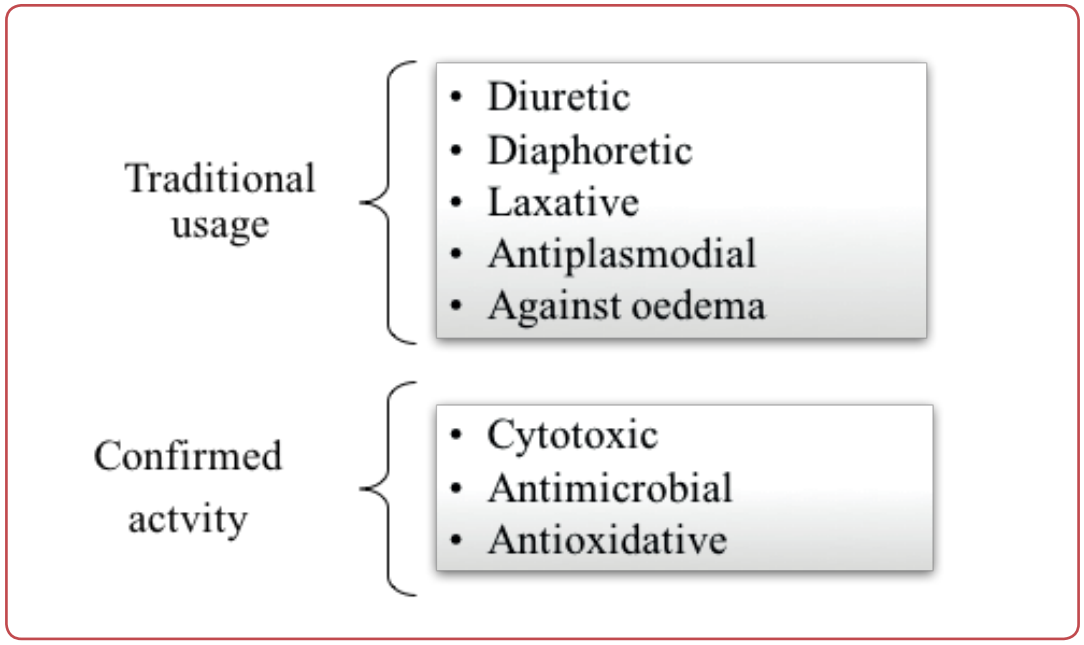


Figure 2: *Ethnopharmacology of E amethystinum and its confirmed pharmacological activity*

Table 2: *Eryngium species used in ethnopharmacology and traditional medicine*

Plant part	Country	Usage	Reference
Eryngium bungei	Iran	Hepatitis	14
Eryngium campestre roots	Turkey	Burns, pains, haemorrhoids and sexual diseases	15
Eryngium caucasicum	Iran	Diuretic, against kidney stones	12
Eryngium creticum root	Jordan	Against snake bites	16
Eryngium foetidum	Sao Tome	Skin changes	17
Eryngium yuccifolium	Mexico	Snake bites, antinociceptive	18

Chemical composition of *E amethystinum* essential oils

Data on the chemical composition of essential oils and extracts of *E amethystinum* were gathered and compared to various species within the genus *Eryngium*. The data sources were *Google Scholar* and *PubMed d*atabases, with keywords selected in accordance with MeSH terminology.

The essential oil of *E amethystinum* aerial parts from Croatia was analysed by Dunkić et al. Total amount of EO identified was 93.1 %, with 42 identified compounds. The methods used were gas chromatography-mass spectometry (GC-MS) and gas chromatography-flame ionisation detector (GC-FID).

The main constituents of essential oil of *E amethystinum* aerial parts were β-caryophyllene (19.70 %), α-pinene (12.30 %), 2,3,6 trimethylbenzaldehyde (7.90 %), α-bisabolol (7.90 %), germacrene D (7.20 %). Among observed fractions, sesquiterpene fraction was the most dominant with 60.00 %. Sesquiterpene hydrocarbons accounted for 40.10 % of total amount of EO, while oxygenated sesquiterpenes accounted for 19.90 %. Afforementioned β-caryophyllene was the most prominent sesquiterpene hydrocarbon with 19.70 %, accompanied with germacrene D (7.20 %), while the most prominent oxygenated sesquiterpenes were α-bisabolol (7.90 %) and γ-eudesmol (5.90 %).

Monoterpene hydrocarbon fraction accounted for 15.20 %, with α-pinene being the most prominent compound (12.30 %). Oxygenated monoterpenes were present in percentage of 5.30 %, with camphor being the most prominent compound (3.10 %). Carbonyl compounds had the percentage of 8.60 %, with 2,3,6 trimethylbenzaldehyde

as the most prominent compound (7.90 %). Phenolic compounds were present in 1.80 %, while alyphatic hydrocarbons accounted for 2.20 %.[19]

EO of *E amethystinum* aerial parts from Croatia was analysed by Kremer et al by GC-MS and GC-FID. Total amount of EO identified was 91.30 % of EO with 63 identified compounds. Sesquiterpene fraction accounted for 54.90 %. Sesquiterpene hydrocarbons were present in amount of 35.20 %, with β-caryophyllene (15.20 %) and germacrene D (5.90 %) as the most prominent compounds. Oxygenated sesquiterpenes accounted for 19.70 %, with γ-eudesmol (6.40 %) as the most prominent compound. Monoterpene hydrocarbons were present with 14.50 %, with α-pinene as the most dominant compound (10.2 %). Carbonyl compounds were present 10.00 % with 2,3,6 trimethylbenzaldehyde as the most prominent compound (9.30 %). Other classes accounted for smaller amounts; phenolic compounds with 2.0 %, hydrocarbons for 1.00 %.[20]

EO of *Eryngium amethystinum* aerial parts from Italy was analysed by Cianfaglione et al. The total amount of identified EO was 88.70 %, with total of 44 identified compounds. Sesquiterpenes were the most dominant fraction with 84.00 %; sesquiterpene hydrocarbons accounted for 76.50 %, while the oxygenated sesquiterpenes took part with 7.50 %. The most prominent sesquiterpene compound was germacrene D (56.7 %). Other fractions were spotted in lesser amounts; monoterpene hydrocarbons accounted for 3.10 %, diterpenes were present in amount of 0.40 %, the percentage of aliphatic compounds was 0.10 %.[21]

EO of *Eryngium amethystinum* aerial parts and roots from Serbia was analysed by Matejić et al by GC-MS and GC-FID. The total amount of identified EO was 99.39 %, with total of 62 identified compounds. Sesquiterpenes were the most dominant fraction with 92.29 %, sesquiterpene hydrocarbons accounted for 65.44 % while the oxygenated sesquiterpenes took part with 26.85 %. The most prominent sesquiterpene compound was germacrene D (23.44 %), it was accompanied with α-gurjunene (10.87 %) and γ-muurolene (8.06 %) Other fractions were spotted in lesser amounts – aldehydes with 1.11 %, ketones with 0.22 %, alcohols with 0.06 % and esters with 3.00 %.[22]

Flamini et al analysed EO from flowers, stem and fruits of *E amethystinum* growing in Italy. The EO of flowers, stem and leaf consisted of 74 identified compounds. The essential oil of *E amethystinum* leaves was rich in germacrene D (31.30 %),[23] double the amount compared to *E vesiculosum* (19.20 %),[24] 2,3,6-trimethylbenzaldehyde and alpha-pinene were also prominent in leaves EO analysed by Flamini et al (24.70 % and 11.80 %, respectively). These compounds were the most prominent compounds in essential oil of *E amethystinum* fruits: α-pinene (17.00 %), followed by 2,3,6-trimethylbenzaldehyde (16.90 %) and germacrene D (7.60 %).[23]

EO of *Eryngium amethystinum* roots from Serbia analysed by Matejić consisted of 34 identified compounds, accounting for 99.84 %; mostly of aromatic aldehydes. The most prominent compounds were 2,3,4-trimethylbenzaldehyde (74.10 %) and 2,3,5-trimethylbenzaldehyde (15.16 %).

Comparison of essential oils composition

Sesquiterpene fraction predominated in all essential oils of *Eryngium amethystinum* aerial parts. The most abundant sesquiterpene fraction was observed in EO from Serbia analysed by Matejić (91.29 %), accompanied by EO from Italy analysed by Cianfagnone et al (84.0 %), EO from Croatia analysed by Dunkić et al (60.00 %) and Kremer et al (54.9 %). Sesquiterpene hydrocarbons were most abundant in EO from Italy (76.5 %)[21] compared to EO from Serbia (65.44 %)[22] and Croatia (40.1 % and 35.2 %, respectively).[19, 20]

The most prominent sesquiterpene compound in EO from Italy and Serbia was germacrene D (56.70 and 23.44 percent, respectively).[21, 22] Spathulenol was detected in EO of *E amethystinum* from Serbia (4.67 %), it had lower percentage compared to *E palmatum* from Serbia (38.61 %).[22] It inhibited the growth of drug-resistant clinical isolates of *Mycobacterium tuberculosis* when tested *in vitro*.[25] β-caryophyllene was the most prominent sesquiterpene compound in both of essential oils from Croatia (19.70 % and 15.20 %, respectively).[19, 20] This amount was higher than in EO of *Eryngium duriaei* subsp. *juresianum* (M Laínz) (6.3 %),[26] but lower compared to EO from *E vesiculosum* aerial parts (20.3 %).[24]

Monoterpene fraction hydrocarbon fraction was

the most abundant in essentials oils of *E amethystinum* aerial parts from Croatia (15.2 % and 14.5 %) [19, 20] while it was presented in small amounts in EO from Italy and Serbia (3.1 and 0.1 %, respectively).[21, 22] α-pinene was also present in essential oil from Croatia (12.30 % and 10.20 %, respectively).[19, 20] This amount is lower compared to *E paniculatum* Cav (19.10 %), [27] but higher than *E yuccifolium* Michaux (7.90 %).[28]

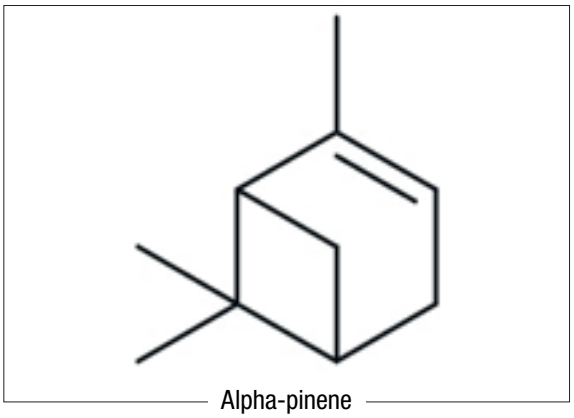
The most prominent compound in essential oils of *E amethystinum* flowers from Italy analysed by Flamini were α-pinene (25.60 %), 2,3,6-trimethylbenzaldehyde (22.00 %) and germacrene D (14.50 %). The essential oils of *E amethystinum* flowers from Italy were rich in aromatic compounds. The most prominent compound was 2,3,6-trymethylbenzaldehyde (22.00 %), accompanied by 2,3,4-trimethylbenzaldehyde (4.40 %). The percentage of 2,3,6-trymethylbenzaldehyde was lower than percentage of 2,4,6-trimethilbenzaldehyde.[23]

The percentage of α-pinene was higher than in *E paniculatum* (19.10 %).[27] The percentage of germacrene D in EO of *E amethystinum* flowers was similar to the percentage of bicyclogermacrene D in *E bourgatii* (15.10 %),[29] but lower than percentage of germacrene D in *E campestre* (30.30–40.30 %).[30]

The essential oil of *E amethystinum* leaves from Italy was rich in germacrene D (31.30 %),[23] double the amount compared to *E vesiculosum* (19.20 %)[24] 2,3,6-trimethylbenzaldehyde and α-pinene were also prominent (24.70 % and 11.80 %, respectively). These compounds were the most prominent compounds in essential oil of *E amethystinum* fruits: α-pinene (17.00 %), followed by 2,3,6-trimethylbenzaldehyde (16.90 %) and germacrene D (7.60 %).[23]

Pharmacological activity of the most prominent compounds

α-pinene (Figure 3) showed anti-inflammatory activity in mouse model by suppression of MAPK and NF-kB pathway in peritoneal macrophages.[31] α-pinene also showed anti-inflammatory, hypoglycaemic and hypolipidemic activity in alloxan-induced diabetic rats.[32] It also enhances locomotor activity in rat model of Huntington disease (as main component of *Ducrosia anethifolia*),[33] possibly due to its prominent antioxidative activity.[34] β-caryophyllene exhibited anti-inflammatory activity,[35] anticonvulsant properties[36] and neuroprotective activity.[37] It also inhibits permeability of brain-blood barrier in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced parkinsonism.[38] β-caryophyllene exhibited cytostatic activity towards several cancer cell lines, such as human colon cancer cells (HCT-116) and human colorectal adenocarcinoma (HT-29). Due to presence of methylene and epoxide functional groups, β-caryophyllene binds to DNA bases and proteins, making itself potential modulator of cell signal pathways in cancer cells.[39] β-caryophyllene enhances activity of cisplatin towards lung cancer cells by regulating cell cycle and inducing apoptosis.[40]



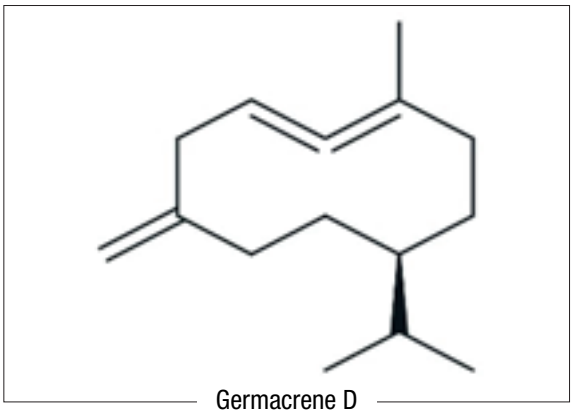


Figure 3: Chemical formulas of the most prominent compounds in E amethystinum essential oils

Phytochemical composition of methanolic extracts of *Eryngium amethystinum*

Vuković et al analysed methanolic extracts of *E amethystinum* aerial parts from the Raška region of southern Serbia. The total amount of 63 compounds was tentatively identified. Among them, there are 15 cinnamic acids derivates and eight hydroxybenzoic acids. Flavonoid fraction consisted of 24 flavonoid derivatives and four aglycones. Coumarin fraction consisted of three identified compounds.[41] The method used was Ultra High-Pressure Chromatography-Mass Chromatography (UPLC- LTQ- Orbitrap-MS).

Polyphenolic acids included syringic acid, glucosyringic acid, protocatechuic acid, caffeic acid, chlorogenic acid, quinnic acid, rosmarinic and cychoric acid. Protocatechuic and rosmarinic acid were present in methanolic extract sample analysed by Kremer et al (0.02 %).

Flavonoid fraction consisted of four aglycones (luteolin, quercetin, kaempherol and isorhamnetin and their 24 derivatives) in methanolic extract analysed by Vuković et al. Rhutin was present in *E amethystinum* methanolic extract analysed by Kremer et al.[20]

Coumarine fraction obtained by Vuković et al consisted of esculetin-6-O-glucoside, Scopoletin-7-O-glucuronide and 4-methylumbeliferon. Other compounds present in this extract were gluconic acids, quinic acid, malic acid, succinic acid, hydroxymethylglutaric acid and their derivatives.

Cytotoxic activity of *Eryngium amethystinu*m essential oils

The essential oil of *E amethystinum* herb from Italy was examined by Flamini et al in order to test its cytotoxic activity. A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was conducted on HTC cells to demonstrate the cytotoxic activity of *E amethystinum* herba essential oil. The essential oil of *E amethystinum* aerial parts exhibited strong cytotoxic activity against many cell lines. The HCT_{116} colon carcinoma cells were the most susceptible to EO, with observed IC_{50} value of 1.65 µg/mL. Cytotoxic activity was also strong on MDA-MB_{231} cells, with observed IC_{50} values of 5.32 µg/mL.[23] All aforementioned results satisfy the criteria for promising cytotoxic agent issued by US plant screening programme. The criterium for cytotoxic agent is IC_{50} lower than 20.00 µg/mL.[42] The main compound of observed essential oil was germacrene D. Human hepatocellular carcinoma (Hep G2), human breast adenocarcinoma (MDA-MB_{231} and MCF-$_7$) and human ductal carcinoma (Hs $_{57}$8T) were sensitive to this EO. β-elemene also exhibited inhibitory activity against non-small lung cell lung cancer cell (NSCLC) cell lines, lung fibroblast and bronchial epithelial cell lines.[43] Several carcinoma cell lines, such as colon carcinoma HCT, murine melanoma $B1_6F_{10}$-Nex2, cervical tumour Siha and non-tumoral HFF cell lines proved sensitive to bicyclogermacrene.[44]

Antimicrobial activity of extracts and essential oils

Inflorescences, leaves and stems of *E amethystinum* from Croatia were extracted by Kremer et al to prepare methanolic extracts. The obtained extracts were examined using two-fold dilution method. *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 10536) and *Mycrosporum gypseum* (MFBF S2) were tested. The leaf, stem and flower methanolic extract proved active against *S aureus*, with MIC (minimal inhibitory concentration) of 0.39-1.32 mg/mL. The stem and leaf extracts were characterised with MIC= 0.39 mg/mL, while the flower extract had MIC of 1.32 mg/mL. The leaf, stem and flower methanolic extracts proved active against *E coli* as well, with a MIC 1.53-1.94 mg/mL. The leaf and flower extracts had MIC of 1.53 mg/mL, while stem extract had MIC of 1.94 mg/mL. Leaf extract of *E amethystinum* exhibited MIC of 0.06 mg/mL, when applied to *Candida albicans*. Clinical isolates of fungal strains exhibited high sensitivity on all three extracts. *C glabrata, C krusei* were sensitive to all examined extracts with a MIC of 0.37 mg/ mL. *Microsporum gypseum* was sensitive to *E amethystinum* flower extract with a MIC of 0.06 mg/ mL, while stem extract exhibited MIC of 0.24 mg/mL; leaf extract demonstrated MIC of 0.16 mg/ mL.[20]

The extracts of *E amethystinum* aerial parts from Serbia were prepared by Matejić et al using four solvents of different polarity and examined by microdilution method. The extracts from *E amethystinum* showed an activity in a range from 0.31-20.00 mg/mL.

Water extract of *E amethystinum* aerial parts exhibited MIC of 10 mg/mL when it was applied to *E faecalis*, *P mirabilis*, *P aeruginosa*, *C albicans*. It exhibited MIC of 20 mg/mL when applied to *S aureus*, *S pyogenes*, *S epidermidis* and *K pneumoniae*. The MIC was above 20.00 mg/mL when extract was applied to *E coli*.

Methanolic extract of *E amethystinum* aerial parts exhibited MIC of 2.50 mg/mL when applied to *S aureus*, *E faecalis, S epidermidis*, *K pneumoniae*, *C albicans*. MIC of 5.00 mg/mL was observed when extract was applied to *P mirabilis* and *P aeruginosa*. The highest MIC was observed in case of *E coli* MIC = 20.00 mg/mL.

Ethylacetate extract demonstrated activity against *K pneumoniae* (MIC = 0.31 mg/mL), *P mirabilis*, *P aeruginosa*, *C albicans*, *S aureus* and *S epidermidis* with MIC = 0.62 mg/mL. *S pyogenes* exhibited MIC > 2.50 mg/mL, while *E faecalis* was characterised with MIC > 5.00 mg/mL.

Acetonic extract exhibited the lowest MIC = 0.62 mg/mL against *K pneumoniae*. The MIC of 1.25 mg/mL was observed in case of *S aureus*, *S epidermidis*. *E fecalis, E coli, P mirabilis* and *Candida albicans* showed MIC of 2.50 mg/mL. The strain most resistant to acetonic extract was *S pyogenes*, with the MIC value of > 10.00 mg/mL.

Essential oils obtained from aerial parts and roots using a Clevenger-type apparatus. were significantly active against *Klebsiella pneumoniae* and *Proteus mirabilis*, with MIC lower than 0.0005 µg/mL. The oils isolated from aerial parts proved to be more active than those isolated from the roots. Spathulenol and germacrene D contributed most to antimicrobial activity of essential oils of *E amethystinum* aerial parts from Serbia (Figure 4).[22]

Matejić et al examined the interaction between the active site of *S aureus* tyrosyl-tRNA synthetase and the most prominent compounds in EO from aerial parts and roots of *E amethystinum*. Spathulenol and germacrene D were the most prominent in EO of the aerial parts, along with octanoic and nonanoic acid. 2,3,4-trimethylbenzaldehyde was the most prominent compound in EO of roots.

Spathulenol from the EO of *E amethystinum* aerial parts proved itself as the best fitting compound. Hydrogen bonds formed between ligand and active site influenced binding of ligand to active site of the enzyme. Spathulenol had the lowest energy of binding (-624.43), establishing hydrogen bond to tyrosine Tyr36 of tyrosyl-tRNA synthetase, with a bond length of 2.81 Å. ReRank and MolDock values confirmed the affinity of spathulenol for binding to tyrosyl-tRNA syntethase. Van der Wals interaction and steric energy influenced the binding energy of the examined compounds. The investigation of Van der Wals values for prominent compounds confirmed these previous findings. Spathulenol and germacrene D had the lowest Van der Wals score values (-338.96 and -239.20, respectively), while the 2,3,4-trimethyl-

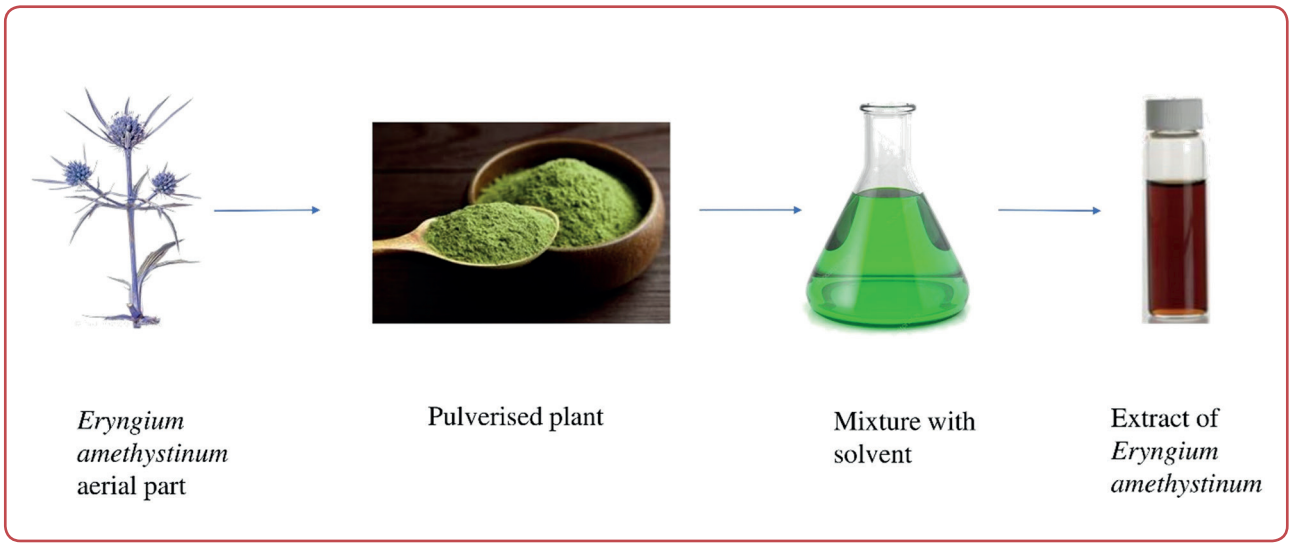


Figure 4: The procedure of obtaining extracts of Eryngium amethystinum

benzaldehyde had the highest values, indicating a higher potential of active compounds to bind to the active site of enzyme compared to the active compounds from roots.[22]

Antioxidative activity of *Eryngium amethystinum* extracts

Oxidative stress is a combination between increased production of reactive oxygen species (ROS) and decreased ability of human organism to neutralise them.[45] In order to protect against the harmful effect of oxidative stress, it is of great significance to find new antioxidative agents. Methanolic and water/methanolic extracts of *E amethystinum* from Poland were examined by Wojtanovski et al using DPPH (2,2-diphenyl-1-picrylhydrazyl) test. The EC_{50} (concentration needed to inhibit 50 % of radicals) value for methanolic extract was 8.12 mg/mL and methanolic-water extracts of *E amethystinum* was 3.51 mg/mL. These values resemble antioxidative activity of beta-carotene, which EC_{50} was 7.85 mg/mL. The activity of these extracts was weaker than the activity of reference compound (Trolox with EC_{50} of 0.08 mg/mL).[46] This study led to another study which combined TLC (thin-layer chromatography) with DPPH assay. Standard activity coefficient (SAC) values were obtained for methanolic-water extract 0.38 and was higher than water-methanolic extracts of *Eryngium planum*, whose value was 0.20, also it was similar to the methanolic extracts (0.11).[47]

Methanolic extracts of *E amethystinum* leaf, stem and flower from Croatia exhibited radical scavenging activity from 30.73 µg/mL-168.78 µg/mL. The antioxidative activity of *E amethystinum* leaf was statistically equal to the activity of BHA (butylated hydroxyanisole). Other tests, such as the beta-carotene-linoleic acid confirmed high antioxidative potential of *E amethystinum* extracts. *E amethystinum* leaf methanolic extracts again proved to be the most active among observed extracts, with an IC_{50} of 90.94 µg/mL.[48]

E amethystinum from Serbia was used to obtain essential oil and extracts of various polarities. Extracts with varying polarity were obtained and their antioxidative activity was examined via DPPH test. The highest activity was recorded in water extract., with IC_{50} of 1.70 mg/mL. 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay also confirmed this result.[22]

The antioxidative activity of essential oil of *E amethystinum* from Italy was examined. DPPH essay showed no activity of *E amethystinum* essential oil. The lack of activity can be linked to high content of sesquiterpene hydrocarbons and oxygenated hydrocarbons. Only a moderate antioxidant power, as indicated by the value of scavenging activity towards ABTS+ radical by *E amethystinum* essential oil (TEAC = 47.12 ± 0.5 µmol TE/g) was observed.[21]

Conclusion

Eryngium amethystinum L is a plant which is used traditionally in Italy and the Balkan countries. Despite its long-standing presence in ethnopharmacology of those countries, this plant remains relatively unexplored. Preliminary research on this topic suggests its potential antimicrobial, antioxidative and cytotoxic potential. All the aforementioned findings provide a solid foundation for further investigation of *Eryngium amethystinum* L from Bosnia and Herzegovina.

Ethics

This study was a secondary analysis based on the currently existing data and did not directly involve with human participants or experimental animals. Therefore, the ethics approval was not required in this paper.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

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