Chemical composition, antimicrobial activities and olfactory evaluations of an essential marjoram oil from Albania as well as some target compounds

Chemische Zusammensetzung, antimikrobielle Aktivitäten und olfaktorische Beurteilungen eines ätherischen Majoranöls aus Albanien sowie einiger Schlüsselkomponenten

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Summary

The chemical composition of an essential marjoram oil from Albania produced for commercial purposes was analysed by GC and GC/MS. 28 volatiles were identified and the following monoterpenes were found as main compounds: terpinen-4-ol (21.33%), *trans*-sabinene hydrate (15.53%), γ-terpinene (14.00%), α-terpinene (8.88%) and sabinene (8.27%). The essential *Origanum majorana* oil, some higher and medium-concentrated constituents, a phenolic reference component and three antibiotics were tested against several microorganisms using modified and well-established and optimised agar dilution and agar diffusion assays to evaluate the antimicrobial activity of the oil as well as these target compounds. In addition, the analytical results were correlated with olfactoric evaluations for reasons of quality control of the essential marjoram oil.

Keywords:
Essential oil, *Origanum majorana* L., chemical composition, antimicrobial activities, olfactoric evaluations

Zusammenfassung

Die chemische Zusammensetzung eines kommerziell hergestellten ätherischen Majoranöls aus Albanien wurde mittels GC und GC/MS analysiert. 28 flüchtige Inhaltsstoffe wurden identifiziert und als Hauptkomponenten die Monoterpene Terpinen-4-ol (21,33 %), *trans*-Sabinenhydrat (15,53 %), γ-Terpinen (14,00 %), α-Terpinen (8,88 %) und Sabinen (8,27 %) gefunden. Die antimikrobielle Aktivität des ätherischen *Origanum-majorana*-Öls sowie einiger Haupt- und Nebenkomponenten wurden mit einem optimierten und bewährten Agardiffusions- und Agarverdünnungstest bestimmt. Zum direkten Vergleich wurden drei Antibiotika sowie eine phenolische Referenzkomponente hinzugezogen. Die analytischen Resultate wurde mit olfaktorischen Bewertungen zur Qualitätskontrolle dieses ätherischen Majoranöles korreliert.

Kennwörter:
Ätherisches Öl, *Origanum majorana* L., chemische Zusammensetzung, antimikrobielle Aktivitäten, olfaktorische Beurteilungen

1 Introduction

In continuation of an international project in the field of combined data interpretation of composition analysis, odour evaluation and antimicrobial activity testings of various aroma samples [1], an essential oil of marjoram (*Origanum majorana* L., syn. *Majorana hortensis* Moench., *Lamiaceae* – common names: English: marjoram, sweet marjoram, knotted marjoram; Arabic: bardagoush, barsagusha, mardqouche, mizunjuske; Dutch: marjolein; French: marjolaine, origane; German: Gartendost, echter Majoran, Meiran, Wurstkraut [2, 3] – from Albania was investigated. Many papers about the composition of *Origanum majorana*, its extracts and essential oils, its utilisation as spice and for flavouring of foodproducts, the applications of these oils in perfumery and cosmetics as well as the biological, pharmacological and toxicological effects as fresh plant, herb, tea, extract and especially essential oil for the treatment of various diseases are available so far [4–18].

This immense number of chemical and antimicrobial data is based on different non-commercial marjoram samples. The aim of this respective study was to analyze an essential oil of *Origanum majorana* from Albania produced under commercial manufacturing conditions by means of GC and GC/MS analysis, olfactory evaluation and antimicrobial testings, using well-established and optimised agar diffusion and agar dilution methods [1].

2 Materials and methods

2.1 Samples and reference compounds

Sabinene hydrate (purity: 98.1%, product no.: 96573) is a product of Fluka-Riedel-de Haen-Sigma Aldrich (Buchs, Switzerland); α-terpinene (85.1%, 22,318-2), γ-terpinene (97.2%, 22,319-0), terpinolene (90.2%, W30,460-3-K), myrcene (90.0%, 276200), (S)-(-)-limonene (95.0%, W504505), and tetracycline hydrochloride (achromycine hydrochloride – 25g, T3383-25G) are products from Sigma-Aldrich Austria Co., Vienna. The essential marjoram oil sample from Albania (800522) was obtained by hydrodistillation from the flowering tops of *Origanum majorana* L. with physico-chemical data: rotation +17.7°, density: 0.892, refraction: 1.4754; and the following reference compounds as terpinen-4-ol (99.1%, 800760), α-terpineol (82%, 800761), linalool (96%, 800506), linalyl acetate (96%, 800507), *p*-cymene (97.0%, 800613), α-pinene (97%, 800079), β-pinene (95%, 800140), β-caryophyllene (97.5%, 800173), eugenol (98.3%, 800316) were purchased from Kurt Kitzing Co., Wallerstein. Ciproxin® 500 mg tablets (1 tablet = 582 mg ciprofloxacine hydrochloride/water) were bought from Bayer Austria Co., Vienna and Lidaprim® infusion bottle (250 mg containing 0.8 g sulfametrol and 0.16 g trimethoprim) from Nycomed Austria Co., Vienna.

2.2 GC analysis

GC/FID analyses were carried out using a GC-14A with split/splitless injector, FID and C-R6A-Chromatopac integrator (Shimadzu, Japan), a GC-3700 with FID (Varian, Germany) and C-R1B-Chromatopac integrator (Shimadzu). The carrier gas was hydrogen (flow rate: 1 mL/min); injector temperature 250 °C; detector temperature, 320 °C. The temperature programme was: 40 °C/5 min to 280 °C/5 min, with a heating rate of 6 °C/min. The columns were 30 m x 0.25 mm bonded DB-5MS fused silica, with a film thickness of 0.50 μm (J & W Scientific Corp., USA) and 30 m x 0.32 mm bonded Stabilwax, with a film thickness of 0.50 μm (Restek, USA). Quantification was achieved using peak area calculations, and compound identification was carried out partly using correlations between retention indices [19–23].

2.3 GC/MS analysis

For GC/MS measurements a GC-17A with QP5050 (Shimadzu, Japan), split/splitless injector and HP-Compaq data system (Hewlett-Packard, USA, Shimadzu GCMSsolution software) and a GC-17A with QP5000 (Shimadzu, Japan), split/splitless injector and Pentium PC data system (HP, USA, Shimadzu class5k software) were used. The carrier gas was helium (flow rate: 1 mL/min); injector temperature 250 °C; interface heating at 300 °C, ionsourceheating at 200 °C, EI-mode was 70 eV, and the scan range was 41–450 amu. For other parameters, see description of GC/FID, above. Mass spectra correlations were done using Wiley, NBS, NIST and our own library as well as published data [1].

2.4 Antimicrobial testings

The essential oil of marjoram, the target compounds and the reference compound were prepared as 20% solutions of ethanol and dissolved in a 0.9% NaCl solution. As test microorganisms (colony-forming units per cm^3 = cfu/cm^3), Gram-positive bacteria *Staphylococcus aureus* ATCC 6538P ($1.5x10^{13}$) and *Enterococcus faecalis* (clinical isolated, $1x10^{13}$), Gram-negative bacteria *Escherichia coli* ATCC 8739 ($1x10^{11}$), *Pseudomonas aeruginosa* G 28 ($2x10^{13}$), *Klebsiella pneumoniae* (clinical isolated, $2x10^{13}$), *Proteus vulgaris* (clinical isolated, $6x10^{13}$) and *Salmonella enterica* subsp. *enterica* (clinical isolated, $3x10^{13}$) as well as the yeast *Candida albicans* ATCC 10231 ($1.9x10^{10}$) – all products from the National Bank of Industrial Microorganisms and Cell Cultures, Sofia, Bulgaria – were used.
The antimicrobial activity was studied by two well-established and optimised methods as follows: agar diffusion disc method using 6 mm paper discs and quantities of 6.0 μL of the sample. After cultivation of the bacteria and the yeast at 37 °C for 24 h the diameter of the inhibition zone (IZ) was measured. Agar serial tube dilution method results in a minimum inhibitory concentration (MIC) as follows: The essential oil, pure and reference compounds were added to a saline solution (0.5% NaCl in distilled water) containing 1.0% (v/v) Tween 80 at the appropriate volumes to produce final concentrations of the samples in the range of 1–1000 ppm. Petri dishes were inoculated by pipetting 6.0 μL of the serial diluted samples, the reference compounds (the tablets of Ciproxin® were added as solution in saline at a quantity of 300 μg) and 0.1 cm^3 of the desired culture on paper discs (6 mm) and then incubated at 37 °C for 24 h.

2.5 Olfactoric evaluations

The essential marjoram oil from Albania was olfactorily evaluated by a professional perfumer and two aroma chemists; the aroma described in *Tab. 1* correlated with odour impressions published [1, 24–29].

3 Results and discussions

The essential oil of *Origanum majorana* L. from Albania was olfactorily evaluated as follows: herbaceous, aromatic-spicy, slightly terpenous, reminding of terpinen-4-ol. By means of gas chromatographic-spectroscopic analysis (GC/FID and GC/MS) of the essential marjoram oil 28 constituents were identified. The chemical composition confirms the authenticity of the sample by comparing retention indices with those of literature data mentioned in *Tab. 1* as well as scientific publica-

Component	RI*	Relative concentration [%]#	Odour§
α-Thujene	930/1025	0.75	herbal, green, weak earthy
α-Pinene	942/1036	0.83	pine-like, warm, terpenous
Sabinene	972/1130	8.27	fresh, fruity, spicy
β-Pinene	979/1122	0.46	resinous-piney, dry-terpenous
Myrcene	988/1158	2.13	weak citrus and lime-like
α-Phellandrene	1002/1173	0.42	citrus-like, weak peppery
p-Cymene	1015/1273	1.29	citrus-like
α-Terpinene	1017/1189	8.88	herbal, weak fruity, terpenous
Limonene	1020/1206	2.03	lemon-like, fresh
β-Phellandrene	1022/1214	2.16	citrus-like, weak herbal-spicy
γ-Terpinene	1057/1250	14.00	citrus-like, herbal, terpenous
cis-Sabinene hydrate	1060/1463	3.80	spicy, weak fruity
Terpinolene	1073/1282	3.20	sweet-piney, terpenous
trans-Sabinene hydrate	1090/1562	15.53	spicy, weak fruity
Linalool	1093/1515	1.35	fresh-floral
cis-p-Menth-2-en-1-ol	1131/1518	2.30	camphoraceous, fresh
Terpinen-4-ol	1168/1624	21.33	fruity, floral, terpenous
α-Terpineol	1181/1717	3.26	floral, lilac-like
cis-Piperitol	1196/1662	0.12	minty
trans-Piperitol	1208/1727	0.46	minty
cis-Sabinene hydrate acetate	1221/1657	0.05	fresh-spicy, weak fruity
Geraniol	1237/1802	0.10	sweet-floral, rose-like, fruity
Linalyl acetate	1243/1542	2.23	floral, weak fruity
trans-Sabinene hydrate acetate	1257/1608	0.08	weak spicy
β-Caryophyllene	1432/1617	2.28	dry, woody-spicy
α-Humulene	1437/1681	0.10	woody, spicy
Bicyclogermacrene	1500/1726	1.38	woody-spicy, weak herbal
Caryophyllene oxide	1576/1989	0.06	warm-spicy, woody
Total		**98.85**	

* *non-polar/polar column*
\# *in relative % peak area using GC-FID with a non-polar column (mean value of 3 analyses)*
§ *in accordance to published data [1, 24–29]*

Tab. 1: Composition and olfactoric evaluation of the marjoram essential oil from Albania.
Tab. 1: Zusammensetzung und olfaktorische Beurteilung des ätherischen Majoranöls aus Albanien.

tions and own databases. The main compounds were as follows (concentration higher than 3.0%, calculated as relative % peak area using GC/FID with a non-polar column, see *Tab. 1*): terpinen-4-ol (21.33%), *trans*-sabinene hydrate (15.53%), γ-terpinene (14.00%), α-terpinene (8.88%), sabinene (8.27%), *cis*-sabinene hydrate (3.80%), α-terpineol (3.26%) and terpinolene (3.20%).
Using olfactoric data of previously published references (see references cited in *Tab. 1*) the characteristic odour of the marjoram sample can be especially correlated to α-thujene, α-terpinene, γ-terpinene and bicyclogermacrene (herbaceous notes), sabinene, α-phellandrene, β-phellandrene, *cis*- and *trans*-sabinene hydrate, *cis*-sabinene hydrate acetate, β-caryophyllene, α-humulene, bicyclogermacrene and caryophyllene oxide (aromatic-spicy notes), α- and β-pinene, α- and γ-terpinene, terpinolene and terpinen-4-ol (principal aroma compound for the odour impression "reminding of terpinen-4-ol", terpenous notes, see *Tab. 1*).
Results of antimicrobial tests (agar diffusion and agar dilution method in accordance to [1]) of the essential marjoram oil from Albania against various microorganisms were as follows (see *Tab. 2*): intermediate to high effects against all Gram-positive (*Staphylococcus aureus* and *Enterococcus faecalis*) and Gram-negative bacteria strains (*Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Klebsiella pneumoniae* and *Salmonella enterica* subsp. *enterica*) as well as the yeast *Candida albicans,* used in these testings. This result is noteworthy, because essential marjoram oil of previous antimicrobial investigations was found not to be effective against all test strains and both agar methods [10, 12–17].

Correlations with the data of high antimicrobial active, phenolic compounds eugenol and the synthetic antibiotics tetracycline hydrochloride and Ciproxin® (these products and Lidaprim® are known to have no effects against the yeast *C. albicans*) prove the effectivity of the chosen testing methods, while Lidaprim® (no effects against *P. aeruginosa* and *K. pneumoniae*) did not show the antimicrobial activity as expected [1].
Antimicrobial tests with pure compounds, identified as main or minor constituents of the essential marjoram oil, such as α- and γ-terpinene, terpinolene, terpinen-4-ol, α-terpineol, linalool, linalyl acetate, *p*-cymene, myrcene, limonene, α- and β-pinene, sabinene hydrate (mixture of *cis*- and *trans*-sabinene hydrate) and caryophyllene showed the following antimicrobial activity (see *Tab. 2*): Only the monoterpene alcohols terpinen-4-ol, α-terpineol and linalool were moderately to highly effective against all strains of Gram-positive and Gram-negative bacteria as well as the yeast, similarly to the marjoram sample. Therefore, these three constituents are of basic importance for the excellent

Sample	*Staphylococcus aureus* 1.5x10^{13} cfu/cm^3		*Enterococcus faecalis* 1x10^{13} cfu/cm^3		*Escherichia coli* 1x10^{11} cfu/cm^3		*Pseudomonas aeruginosa* 2x10^{13} cfu/cm^3		*Proteus vulgaris* 6x10^{13} cfu/cm^3		*Klebsiella pneumoniae* 2x10^{13} cfu/cm^3		*Salmonella enterica subsp. enterica* 3x10^{12} cfu/cm^3		*Candida albicans* 1.9x10^{10} cfu/cm^3	
	IZ mm	MIC ppm	IZ mm	MIC ppm	IZ mm	MIC ppm	IZ mm	MIC ppm	IZ mm	MIC ppm	IZ mm	MIC ppm	IZ mm	MIC ppm	IZ mm	MIC ppm
Marjoram oil	27	60	13	60	11	6	7	600	18	60	9	60	8	600	30	60
α-Terpinene	13	600	17	60	10	600	-*	-	8	600	9	600	-	-	10	60
γ-Terpinene	-	-	8	600	-	-	-	-	-	-	-	-	-	-	12	60
Terpinolene	10	600	14	60	12	60	-	-	-	-	8	600	8	600	17	60
Terpinen-4-ol	22	60	14	60	35	60	10	60	15	60	22	60	12	60	25	6
α-Terpineol	18	6	17	6	28	6	15	6	17	6	22	6	18	6	35	6
Linalool	17	60	12	60	26	60	8	60	8	6	15	6	8	60	27	6
Linalyl acetate	30	6	28	60	-	-	-	-	-	-	-	-	8	600	8	600
p-Cymene	9	600	10	600	-	-	-	-	-	-	-	-	-	-	18	600
Myrcene	-	-	-	-	7	600	-	-	8	600	-	-	-	-	-	-
Limonene	9	60	8	60	7	6	-	-	7	60	-	-	9	60	15	60
α-Pinene	12	60	10	60	10	60	8	600	-	-	8	600	8	600	20	6
β-Pinene	10	600	10	60	8	600	-	-	8	600	-	-	-	-	10	60
Sabinene hydrate	8	60	7	60	7	60	-	-	7	6	-	-	7	6	10	60
Caryophyllene	13	60	16	60	8	600	-	-	8	600	8	600	8	600	7	6
Eugenol	15	600	20	600	16	600	25	600	28	600	20	600	27	600	11	600
Ciproxin®	35	60	33	60	22	60	32	600	25	600	25	600	10	600	-	-
Lidaprim®	27	60	27	60	11	60	-	-	23	60	-	-	8	60	-	-
Tetracycline hydrochloride	15	60	22	60	11	600	15	600	16	600	20	60	10	600	-	-

** no inhibition observed*

Tab. 2: Antimicrobial activities of the essential marjoram oil from Albania, some main and minor compounds as well as reference compounds.
Tab. 2: Antimikrobielle Aktivität des ätherischen Majoranöls, einiger Haupt- und Nebenkomponenten sowie der Vergleichssubstanzen.

antimicrobial properties of the essential *O. majorana* oil from Albania. All other tested compounds (without γ-terpinene against *S. aureus* and myrcene against both Gram-positive bacteria) were found to have a medium to high effectivity against the Gram-positive bacteria and the yeast *C. albicans* (only myrcene did not show any activity) and therefore may act with the above-mentioned monoterpene alcohols in a synergistic way. A general statement concerning the effectivity of the pure compounds (without terpinen-4-ol, α-terpineol and linalool) against the Gram-negative bacteria cannot be given, because these components were not active against one (caryophyllene) or more strains.

In conclusion, we can report that the essential *Origanum majorana* oil from Albania of our interest was especially rich in terpinen-4-ol, *trans*-sabinene hydrate and γ-terpinene, and showed medium to high antimicrobial activities against all strains of Gram-positive and Gram-negative bacteria as well as the yeast *Candida albicans*, used in these testings. The monoterpene alcohols terpinen-4-ol, α-terpineol and linalool, as main and minor constituents of this marjoram sample, were especially responsible for these effects. Further compounds of the essential *O. majorana* oil may act in a synergistic way against Gram-positive bacteria and the yeast *C. albicans* (as reported for e.g. origanum oil [18]).

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