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CHEMICAL, ANTIOXIDANT AND ANTIMICROBIAL ANALYSIS OF THE ESSENTIAL OIL AND EXTRACT OF *ARTEMISIA ALBA* TURA

SOFIJA ĐORĐEVIĆ[a,*], DRAGANA STANISAVLJEVIĆ[b], MIHAILO RISTIĆ[a], MARINA MILENKOVIĆ[c], DRAGAN VELIČKOVIĆ[b], SAŠA STOJIČEVIĆ[d], BRANISLAV ZLATKOVIĆ[e]

[a]*Institute for Medicinal Plant Research "Dr Josif Pančić", 1 Tadeuša Košćuška St., 11000 Belgrade, Serbia*
[b]*College of Agriculture and Food Technology, 1 Ćirila i Metodija St., 18400 Prokuplje, Serbia*
[c]*Department of Microbiology and Immunology, Faculty of Pharmacy, University of Belgrade, 450 Vojvode Stepe St., 11000 Belgrade, Serbia*
[d]*Faculty of Technology, University of Niš, 124 Bulevar oslobodjenja St., 16000 Leskovac, Serbia*
[e]*Faculty of Agriculture, University of Belgrade, 6 Nemanjina St., 11080 Zemun, Belgrade, Serbia*

This study examines the essential oil and the ethanol extract of *Artemisia alba* Turra herb, in terms of chemical composition, antioxidant and antimicrobial activities in order to select the active pharmacological isolate for making phytopreparation from the group of bitter aromatics and/or bitter liqueurs. Chemical composition of the essential oils and volatile fractions of the extract was determined by GC-FID/GC-MS and non-volatile fractions of the extract by HPLC. The content of total phenolics and flavonoids in the extracts was determined by Folin-Ciocalteu reagent and aluminum chloride, respectively. The antioxidant activity of isolates was tested by FRAP and DPPH tests and the antimicrobial by microdilution method. Major constituents in the essential oil were camphor, artemisia ketone and 1,8-cineole (23.7%, 15.2% and 14.1%, respectively), and in the volatile fraction of the extract, scopoletin (14.0%) and corymbolone (10.3%). The main components in non-volatile fraction of the extract were kaempferol 3-O-(6"-O-malonylglucoside)-7-O-rhamnoside, chlorogenic acid and rutin (16.1%, 11.4% and 9.5% respectively). The content of total phenolics in the extract, expressed as gallic acid was 77.18 mg/g and flavonoids, expressed as rutin 53.80 mg/g of dry extract. Extract exhibited stronger antioxidant activity than essential oil and better antimicrobial activity on a larger number of tested microorganisms.

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Keywords: Artemisia alba Turra, essential oil, extract, GC-MS, HPLC, antioxidant, antimicrobial activity

1. Introduction

Amara pura and *Amara aromatica* are a special group of bitter and bitter aromatic herbal drugs, which cause the secretion of saliva and enzymes in the digestive tract by its bitterness, when applied *per os*. They are used to increase appetite in anorexia, for better digestion of food (in dyspepsia, reduced secretion of digestive enzymes, and flatulence), and they are often applied as

*Corresponding author: sdjordjevic@mocbilja.rs

cholagogues. These drugs do not only improve the appetite, but are also used as tonics and roborants, which strengthen the body (children, elderly and sick people). The best-known drugs are bitter gentian, centaury, yarrow, bitter orange peel, wormwood, etc. [1,2].

According to the European Commission's Concerted Action on Functional Food Science in Europe (FuFoSE), food is not meant only to provide the necessary nutrients, but also to prevent nutrition-related diseases and improve physical and mental health of consumers. In this sense, functional food has a very important role. Herbal isolates in functional products have different pharmacological activities (antioxidant, antimicrobial, anti-inflammatory etc.) and they affect the sensory properties of food and beverage [3]. Products based on extracts of bitter aromatic plants (sweet and bitter liqueurs, special liqueurs and dessert liqueur drinks) are numerous on the market. In addition to specific pleasant aroma, they are also used to reduce stress, fatigue, and regulate digestion [4,5].

This plant, belonging to the genus *Artemisia*, Asteraceae family is entitled as *Artemisia alba* Turra in European Flora, and as *A. lobelii* All (syn. *A. camphorata* Vill., *A. alba* Turra) in Serbian Flora [6,7]. Genus includes over 200 species, mostly inhabiting the steppe regions of Europe, Asia, North and Central America. *Artemisia* species are widely used in traditional medicine of many countries. In addition to the fact that they belong to the pharmacological group of bitter aromatics, plants of this genus also exhibit anti-inflammatory, antioxidant, antifungal, anthelminthic and antitumor activity [8-10]. Isolates of *A. mexicana*, *A. princeps*, *A. diffusa*, *A. oliveriana*, *A. scoparia*, *A. turanica*, *A. dracunculus* exhibit significant antimicrobial activity [11-14]. Essential oils of species *A. scoparia* and *A. capillaries* display antibacterial activity on 15 different strains of bacteria which commonly cause mouth infections [15]. Some *Artemisia* species, due to the presence of terpenes, are used as natural herbicides [16]. *Artemisia* species contain significant amounts of polyphenolic compounds [17]. Composition of the essential oils of the genus *Artemisia* varies depending on the habitat, the climate and the way of drying the plant material [18-22].

To our knowledge, ethanol-water extracts of *A. alba* herb have not been studied in terms of chemical composition and biological activity. Bitter aromatic phytopreparations and bitter liqueurs that include isolates of this species as one of the ingredients are not present neither on domestic nor foreign markets. Therefore, we focused our research on the comparative chemical and biological analysis of the essential oil and ethanol-water extract of *A. alba* from the locality of Knjaževac (South-East Serbia) in order to select pharmacologically active isolate as potential raw material for making bitter aromatic phytopreparations and/or bitter liqueurs.

2. Experimental

2.1. Chemicals and reagents

All chemical substances used in the experimental work were of analytical purity: Folin-Ciocalteu reagent (FC), 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid and rutin (Sigma Chemical Company, USA), sodium carbonate, aluminum chloride hexahydrate, 2,4,6-tripyridyl-*s*-triazine (TPTZ reagent), vitamin C, iron(III) chloride hexahydrate (Merck, Germany), Müller-Hinton and Sabouraud broth (Torlak, Serbia). Standards for HPLC were obtained from Sigma (St. Louis, MO, USA). HPLC grade methanol was purchased from Merck (Darmstadt, Germany).

2.2. Plant materials, preparation of essential oil and extract

Herb of *A. alba* was collected during the full flowering period, in August 2009, at the site of Golemi kamen, Knjaževac (South-East Serbia) and it was naturally dried and pulverized. Voucher specimen BEOU-16474 have been deposited at the Herbarium of the Institute of Botany and Botanical Garden "Jevremovac", Faculty of Biology, University of Belgrade. Isolation of essential oil: The essential oil was isolated by distillation of plant material in the Clevenger type apparatus [23] and kept at +4 ° C. Preparation of the extract: The plant material was extracted by modified procedure of pharmacopoeia single percolation with 70% V/V ethanol [23]. The ratio of obtained mixture of liquid extract according to the amount of used herb was 2:1. Liquid extract

was used for GC analysis. For all other analyzes dry extract was used. The dry extract was prepared from a portion of the liquid extract by evaporation at 50°C, and by drying in a vacuum oven at the same temperature. Finally, it was grinded into a fine powder and stored at +4°C. The yield of extractive substances was determined by gravimetric method.

2.3. Chemical analysis

Chemical analysis of essential oils and volatile fractions of the extract was performed by GC-FID and GC-MS. Identification and quantification of phenolic compounds in the extract (non-volatile fraction) was determined by HPLC. The content of total phenolics and flavonoids in the extract was determined by spectrophotometric method with FC reagent and complexation with aluminum chloride, respectively.

2.3.1. GC-FID and GC-MS analysis

GC-FID analysis was carried out on a Hewlett-Packard, model 5890 II gas chromatograph, equipped with split-splitless injector and automatic liquid sampler (ALS), attached to HP-5 column (25 m · 0.32 mm, 0.32 μm film thickness) and fitted to flame ionisation detector (FID). Carrier gas flow rate (H_2) was 1 mL/min, injector temperature was 250°C, detector temperature 280°C, while column temperature was linearly programmed from 40-260°C (at rate of 4°/min), and held isothermally at 260°C next 10 minutes. Solutions of essential oil in ethanol (~1%), or undiluted extract were consecutively injected by ALS (1 μL, split mode, 1:30). Area percent reports, obtained as result of standard processing of chromatograms, were used as base for the quantification purposes.

The same analytical conditions as those mentioned for GC-FID were employed for GC-MS analysis, along with column HP-5MS (30 m · 0.25 mm, 0.25 μm film thickness), using HP G 1800C Series II GCD system [Hewlett-Packard, Palo Alto, CA (USA)]. Instead of hydrogen, helium was used as carrier gas. Transfer line was heated at 260°C. Mass spectra were acquired in EI mode (70 eV), in m/z range 40-450. Sample solutions were injected by ALS (1 μL, split mode, 1:30).

The constituents were identified by comparison of their mass spectra to those from Wiley275 and NIST/NBS libraries, using different search engines. The experimental values for retention indices were determined by the use of calibrated Automated Mass Spectral Deconvolution and Identification System software (AMDIS), compared to those from available literature [24] and used as additional tool to approve MS findings.

2.3.2. HPLC analysis

Analysis of phenolic compounds in the extract was performed by HPLC method. The HPLC analyses were performed on an Agilent 1100 Series HPLC-DAD system consisting of micro vacuum degasser, binary pump, thermostated column compartment and variable wavelength detector. Column: Agilent Eclipse XDB-C18 4.6 mm IDx150 mm (5 μm). The mobile phase was composed of solvent (A) 0.15% (w/v) phosphoric acid in H_2O:MeOH (77:23, v/v, pH = 2) and solvent (B) methanol as follows: isocratic 0-3.6 min 100% A + 0% B; linear gradient in 24 min 80.5% A+19.5% B; isocratic with 80.5% A+19.5% B up to 30 min; linear gradient in 60 min 51.8% A+48.2% B; linear gradient in 67.2 min 0% A+100% B; followed by isocratic elution with 100% B for last 5 min. Flow rate was 1 mL/min, temperature 15^{o}C. Peak detection in UV region at 350 nm was used [25, 26]. For this analysis 15 μL of liquid extract, concentration of 10 mg/mL, was injected.

2.3.3. Determination of total phenol contents

Total phenol content in the extract was determined according to FC reagent [27, 28], using gallic acid as the standard. The extract solution in 70 % ethanol (0.2 mL, 1 mg/mL) was mixed with the FC reagent (1 mL) and an aqueous solution of Na_2CO_3 (0.8 mL, 7.5 %). After 30 minutes of incubation at room temperature, the absorbance of the reaction compound was measured at 765

nm by the VARIAN Cary-100 spectrophotometer. The overall phenol content was expressed as mg of gallic acid equivalents (GAE)/g of dry extract, and calculated using the equation of the standard curve given below.

$$\text{Absorbance}\,(765\ \text{nm}) = 7.2328\, c_{\text{gallic acid}}\,(\mu g / mL) - 0.2286\,;\ R^2 = 0.9919$$

2.3.4. Determination of total flavonoid contents

The content of total flavonoids in the extract was determined by spectrophotometric method, which is based on the production of complex compounds of flavonoids with aluminum chloride [29]. Plant extract (2.0 mL, 1.0 mg/mL) in 70 % ethanol was mixed with 0.10 mL of (10 %) aluminum chloride, 0.10 mL of (1.0 mol/L) potassium acetate and 2.8 mL of distilled water. After 30 min of incubation at room temperature, the absorbance of the reaction mixture was measured at 415 nm in relation to distilled water. Rutin was chosen as the standard and the total flavonoid content was expressed as mg of rutin equivalents (RE)/g of dry extract, and calculated using the equation of the standard curve given below.

$$\text{Absorbance}\,(415\ \text{nm}) = 12.722\, c_{\text{rutin}}\,(\mu g / mL\ ) + 0.0034\,;\ R^2 = 0.9994$$

2.4. Bioassays

2.4.1. The antioxidant activity of essential oil and extract

The antioxidant activity was determined by measuring the total antioxidant activity which is based upon reduction of Fe^{3+}-2,4,6-tris-(2-pyridyl)-*s*-triazine complex (Fe^{3+}-TPTZ) in acidic conditions (FRAP test), as well as their ability to neutralize DPPH-radical (DPPH test).

FRAP assay

The antioxidant activity of essential oil and extract was determined by Ferric Reducing Antioxidant Power (FRAP) assay [30]. The calibration curve for the determination of the antioxidant capacity was obtained by measuring the absorbance of the series of standard water solutions of Fe^{2+} in the concentration range from 0.20 to 1.00 mmol Fe^{2+}/L. A total of 0.10 mL of essential oil in 70 % ethanol of 0.05 mL/mL concentration (0.10 mL of extract in 70 % ethanol of 0.2 mg/mL concentration) and 3 mL of freshly prepared FRAP reagent (acetate buffer + TPTZ reagent + $FeCl_3x6H_2O$ in the relation 10:1:1) were added in the test tube. After incubation at 37 °C the absorbance was measured at 593 nm. FRAP values were expressed as mmol Fe^{2+}/g of the dry extract and the essential oil.

DPPH assay

The antioxidant activity of the essential oil and the extract was determined by DPPH assay [31,32]. A series of essential oil solutions 0.5 to 25.0 μL/mL (six different concentrations), and extract solutions 0.01 to 1.0 mg/mL (five different concentration), were made in 70 % ethanol, and DPPH radicals in concentration of 0.3 mmol/L in 70 % ethanol for the purpose of determination of antioxidant activity. A total of 1 mL of the DPPH radical solution and 2.5 mL of essential oil and extract solutions of different concentrations were mixed together. After 30 minutes of incubation at room temperature in the dark, the absorbance was measured at 517 nm by the VARIAN Cary-100 spectrophotometer. The capacity for neutralizing free radicals was calculated according to the following equation, where A_s is the absorbance in the presence of essential oil or plant extract in DPPH solution, A_c is the absorbance of the control solution (containing only DPPH) and, A_b is the absorbance of the sample essential oil or extract solution without DPPH.

The capacity of neutralizing of DPPH radicals, (%) = $100 - [(A_s - A_b) \times 100/A_c]$

EC_{50} value was calculated according to the experimental data by the use of the sigmoidal non-curve method and SigmaPlot 2000 Software. EC_{50} values for vitamin C, which was used for comparison, were obtained in the same way as for the essential oil and herbal extracts in the range of concentrations from 0.001 to 0.1 mg/mL.

2.4.2. In vitro antimicrobial activity of essential oil and extract

Broth microdilution method was used to determine the antimicrobial activity of isolates *in vitro* [33]. We used the standard collection of microorganisms from ATCC and NCIMB. The study was conducted against 9 standard strains of bacteria: Gram (+) bacteria *Micrococcus luteus* ATCC 9341, *Micrococcus flavus* ATCC 10240, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212 and *Bacillus subtilis* ATCC 6633); Gram (–) bacteria *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* NCIMB 9111, *Pseudomonas aeruginosa* ATCC 27853 and two standard strains of yeast (*Candida albicans* ATCC 10259 and ATCC 24433).

The minimum inhibitory concentration (MIC) of isolates was determined by broth microdilution test in plates for microtitration with 96 seats. We used 18 hours cultures of mentioned strains (density 5×10^5 cfu/mL) in Mueller-Hinton broth supplemented with Tween 80 (final concentration 0.5% v/v). The oil was dissolved in DMSO and concentrations of 1.56-200 μL/mL were tested. The dry extract was dissolved in DMSO and it was tested in the concentration range 0.81-30 mg/mL. Positive control of bacterial growth was set in the test as well as the sterility control of essential oil and extract. Plates were incubated in aerobic atmosphere for 24 hours at 37 °C for bacteria and 48 h at 26 °C for fungi. Subsequently, the growth of bacteria and yeast was recorded semiquantitatively as turbidity of the medium and pellet at the bottom of the wells.

2.5. Statistical analysis

All measurements were performed in triplicate, and the results were presented as the mean ± standard deviation. Statistical analysis was performed using Microsoft Excel 2000, Origin 7 and Sigma Plot 2000 program.

3. Results

The results of determination of chemical composition of essential oil and ethanol extract of herb *A. alba*, antioxidant and antimicrobial activity, are shown in Tables 1-4.

3.1. GC-FID and GC-MS analysis of essential oil and extract

Content of the oil from the herb of *A. alba* was 5 mL/kg, which is in good agreement with earlier findings dealing with other examined *Artemisia* species (3-5 mL/kg) [9,34].

Results of GC analysis of tested plant isolates are presented in Table 1.

Table 1. The chemical composition of the essential oil (EO) and the extract (EX) of A. Alba

Constituents	KIE	KIL	% EO	% EX
1. tricyclene	927.3	921	0.1	0.2
2. α-pinene	932.0	932	0.8	-
3. camphene	945.9	946	3.2	0.6
4. β-pinene	964.0	974	0.2	1.1
5. yomogi alcohol	1003.0	999	1.8	-
6. *p*-cymene	1024.8	1020	0.4	-
7. β-phellandrene	1016.5	1025	-	0.9
8. 1,8-cineole	1030.8	1026	14.1	5.9
9. artemisia ketone	1063.1	1056	15.2	2.9
10. artemisia alcohol	1086.2	1080	1.3	0.6
11. *trans*-chrysanthenol	1116.2	1096	8.6	7.4
12. chrysanthenone	1126.2	1124	0.3	1.0
13. *trans*-pinocarveol	1140.0	1135	1.5	-

Constituents	KIE	KIL	% EO	% EX
14. camphor	1144.0	1141	23.7	4.3
15. pinocarvone	1163.3	1160	1.6	0.4
16. *cis*-chrysanthenol	1164.4	1160	1.1	-
17. borneol	1167.3	1165	1.4	1.0
18. *cis*-pinocamphone	1174.5	1172	-	1.3
19. terpinene-4-ol	1178.1	1174	1.0	-
20. n-decanal	1216.8	1201	-	1.8
21. bornyl acetate	1287.3	1287	-	0.8
22. *p*-vinyl-guaiacol	1319.3	1309	-	2.0
23. δ-elemene	1348.8	1335	-	1.7
24. methyl eugenol	1409.8	1403	-	0.2
25. *cis*-threo-davanafuran	1418.5	1414	-	0.4
26. *trans*-caryophyllene	1419.2	1417	-	1.6
27. allo-aromadendrene	1461.0	1458	0.5	-
28. γ-muurolene	1481.5	1478	0.6	-
29. germacrene D	1481.7	1484	-	2.9
30. bicyclogermacrene	1497.1	1500	-	0.9
31. α-muurolene	1508.7	1500	-	0.7
32. silphiperfolan-6-α-ol	1506.0	1507	0.5	-
33. 1-endo-bourbonanol*	1523.5	1518	3.3	-
34. silphiperfol-5-en-3-ol B	1536.4	1534	0.7	-
35. silphiperfol-5-en-3-one B	1548.9	1550	1.2	-
36. silphiperfol-5-en-3-ol A	1557.0	1557	1.5	-
37. silphiperfol-5-en-3-one A	1574.2	1574	6.4	0.2
38. spathulenol	1579.7	1577	-	0.3
39. caryophyllene oxide	1584.2	1582	1.6	0.8
40. α-eudesmol	1656.2	1652	-	1.2
41. α-bisabolol	1687.0	1685	6.1	1.9
42. solavetivone*	1691.2	n/a	1.2	-
43. iso-longifolol	1729.7	1728	-	0.4
44. cyclocolorenone	1752.9	1759	-	2.1
45. corymbolone	1853.2	n/a	-	10.3
46. umbeliferone(skimmetine)	1881.5	1875	-	4.9
47. scopoletin	1974.3	n/a	-	14.0
48. ethyl hexadecanoate	1998.2	1992	-	2.2
49. *E*,*E*-geranyl linalool	2050.6	2026	-	2.0
50. phytol [d]	2117.0	2114	-	3.2
Sum of contents %			100.0	84.1
Number of constituents			28	36

KIE - Kovats (retention) index experimentally determined (AMDIS), KIL - Kovats (retention) index - literature data (Adams, 2007), * - tentative identification.

The essential oil contains mainly monoterpenoids and sesquiterpenoids (in the ratio 3:1, approximatelly). Compounds from the class of oxygenated monoterpenes dominate as follows: camphor (23.7%), artemisia ketone (15.2%), 1,8-cineole (14.1%), and *trans*-chrysanthenol (8.6%). Monoterpene hydrocarbons, with camphene as the dominant component (3.2%), are present among other classes of terpenoids, while silphiperfol-5-en-3-one A (6.4%) and α-bisabolol (6.1%) are dominant sesquiterpenoids.

The yield of dried extract was 16.5 g/100 g of dry herb. Thirty-six out of 49 registered components, comprising 84.1% of total composition of the volatile fraction, were identified in the extract (Table 1). The most abundant is scopoletin (14.0%), followed by corymbolone (10.3%), *trans*-chrysanthenol (7.4%) and umbelliferone (4.9%). Since the content of *trans*-chrysanthenol

was almost the same in both type of isolates, the contents of camphor (4.3%), 1,8-cineole (5.9%) and artemisia ketone (2.9%) were lower in the extract than in the oil.

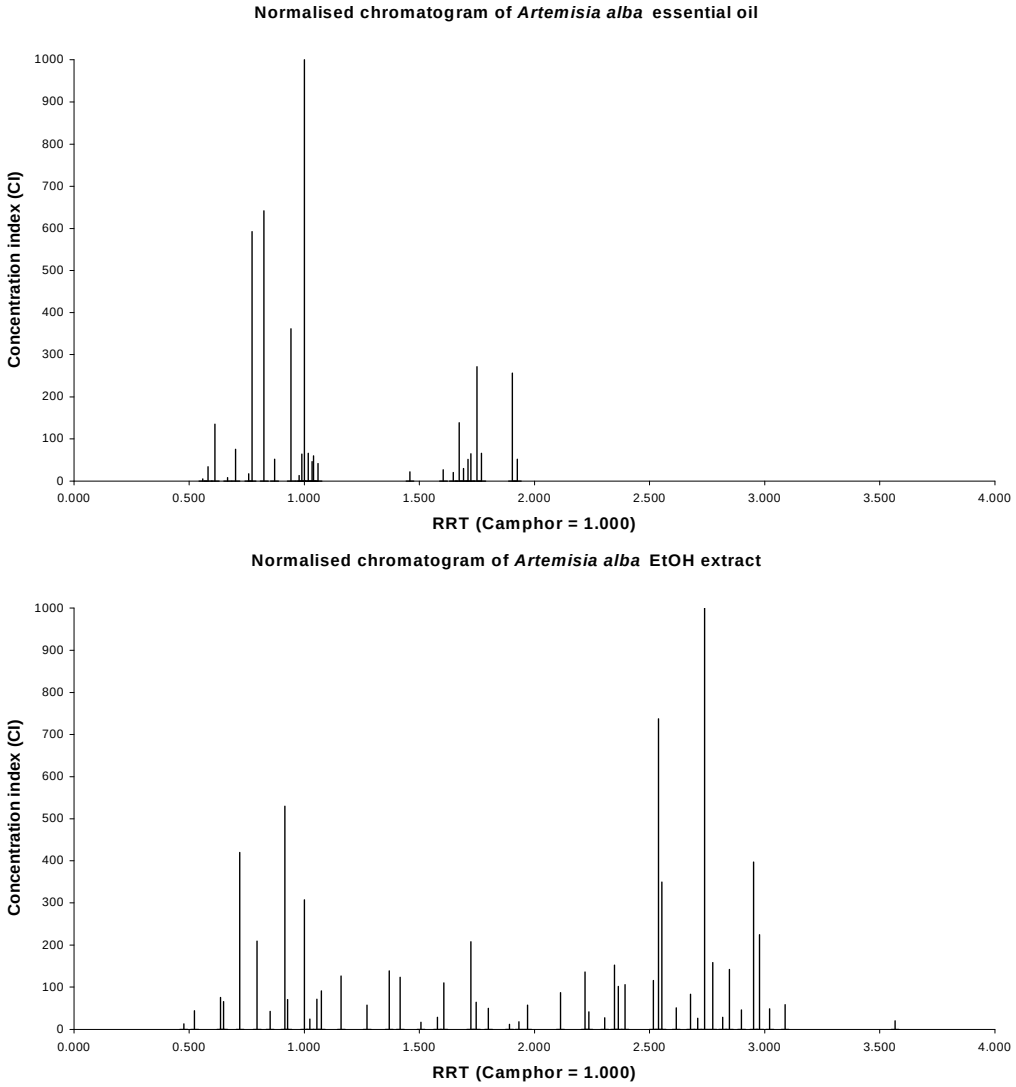

Fig. 1. Chromatographic profiles (GC) of A. alba essential oil and extract.

From chromatographic profiles given in Figure 1 is obvious that composition of the volatile fraction of the extract is more complex than that of the essential oil.

3.2. HPLC analysis of the extract

The results of HPLC analysis of the extract are shown in Table 2.

Table 2. The results of HPLC-DAD analysis of extract of A. alba

No.	Rt (min)	Compounds	% m/m [a]
1	1.88	Gallic acid	0.3
2	8.99	Chlorogenic acid	11.4
3	17.86	Kaempferol 3,7-O-diglucoside	8.4
4	28.66	Luteolin 5-O-glucoside	1.2
5	30.12	Kaempferol 3-O -(6”-O-malonylglucoside)-7-O-rhamnoside	16.1
6	34.38	Rutin	9.5
7	38.73	Luteolin 5-O-(6”-O-malonylglucoside)	7.7
8	42.12	Genkwanin 5-O-glucoside	2.1
9	43.48	Kaempferol 3-O-(6”-O-malonylglucoside)	3.6
10	48.29	Kaempferol 3-O-rhamnoside	3.7
11	52.63	Luteolin	3.4
12	53.24	Kaempferol 7-O-rhamnoside	1.9
13	58.52	Apigenin	3.8

[a] (relative shares in % in relation to the integrated peaks)

Chromatographic profile (HPLC) of *Artemisia alba* extract is shown in Figure 2.

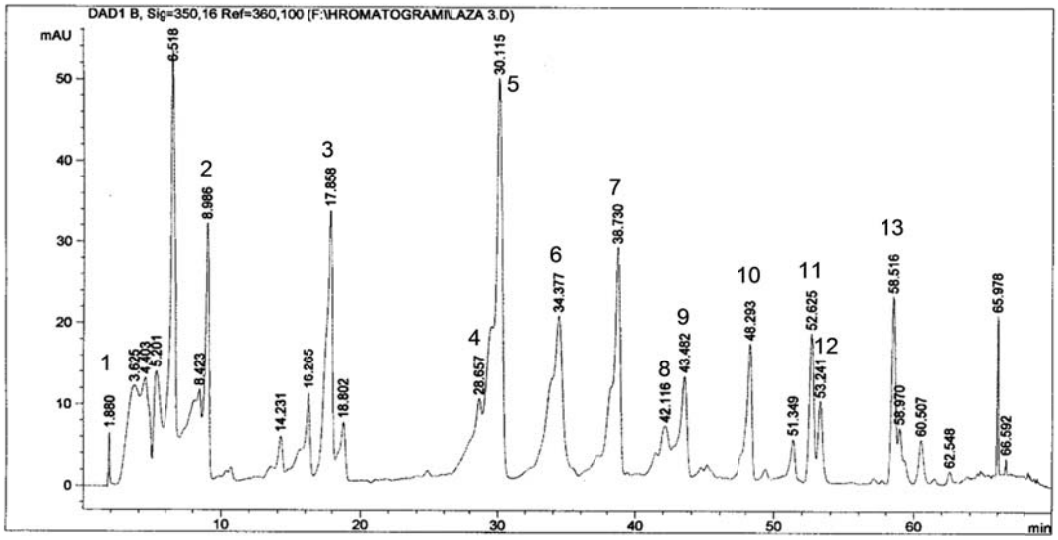

Fig. 2. Chromatographic profile (HPLC) of Artemisia alba extract.

Kaempferol derivatives are present in a larger number and amount than luteolin ones, along with large content of chlorogenic acid. The dominant phenolic component is kaempferol 3-O-(6"-O-malonylglucoside)-7-O-rhamnoside. Kaempferol is also a major flavonol in six *Artemisia* species examined [17].

3.3. Determination of total phenolics and flavonoids in the extract

The content of total phenolics and flavonoids in the extract was 77.18 ± 2.76 mg/g (calculated as gallic acid) and 53.80±0.75 mg/g of dry extract (calculated as rutin) (Table 3).

3.4. The antioxidant activity of isolates

Antioxidant activity of extract and essential oil was determined by FRAP and DPPH test. Results are presented in Table 3. Extract showed better antioxidant activity (FRAP assay 1.16 ±

0.08 mmol Fe^{2+}/g, DPPH assay, EC_{50}, 0.032 ± 0.002 mg/mL) compared to the essential oil (FRAP assay 0.023 ± 0.00 mmol Fe^{2+}/g, DPPH assay, EC_{50}, 14.08 ± 1.09 mg/mL).

Table 3. Content of the total phenols and flavonoids, and the antioxidant activities of tested isolates

Samples	Total phenols	Total flavonoids	FRAP assay	DPPH assay (EC_{50})
	mg GAE/g of dry extract	mg RE/g of dry extract	mmol Fe^{2+}/g	mg/mL
Essential oil	-	-	0.023±0.00	14.08±1.09
Extract	77.18±2.76	53.80±0.75	1.16±0.08	0.032±0.00
Vitamin C	-	-	-	0.011±0.00

The antioxidant activity of vitamin C (EC_{50}=0.011 ± 0.00 mg/ml) is tree times higher compared to the extract.

3.5. Antimicrobial activity of isolates

The results of antimicrobial activity of tested isolates of *A. alba* to the standard microorganisms, was determined by microdilution test and presented in Table 4.

Table 4. Antimicrobial activity of essential oil and extract of A. alba to standard test microorganisms

Microorganism	EO (μL/mL)	EX (mg/mL)
Gram (+) bacteria		
Micrococcus luteus ATCC 9341	3.1	1.6
Micrococcus flavus ATCC 10240	25.0	0.8
Staphylococcus aureus ATCC 25923	12.5	1.6
*Staphylococcus epidermidis*ATCC12228	3.1	3.2
*Enterococcus faecalis*ATCC 29212	6.2	7.5
*Bacillus subtilis*ATCC 6633	3.1	7.5
Gram (–) bacteria		
Escherichia coli ATCC 25922	6.2	7.5
Klebsiella pneumoniae NCIMB 9111	12.5	7.5
Pseudomonas aeruginosa ATCC 27853	25.0	7.5
Fungi		
*Candida albicans*ATCC 10259	25.0	7.5
*Candida albicans*ATCC 24433	25.0	7.5

EO=essential oil; EX=extract

Extract and essential oil exhibit significant antimicrobial activity. The extract is more active against majority of tested microorganisms (seven out of eleven). It is especially active against G (+) bacteria *M. luteus*, *M. flavus* and *S. aureus*.

The essential oil demonstrated stronger activity against *S. epidermidis*, *E. faecalis*, *B. subtilis* and *E. coli*. Extract showed higher antifungal effect in relation to the essential oil.

4. Discussion

In this paper, we showed that *A. alba* herb isolates prepared by different procedures, have different chemical composition, which causes different level of biological activity.

Chemical composition of the tested essential oil from the site of Knjaževac is partially consistent with the results of previous studies of this type of oil from Serbia [19,34]. Namely, Stojanović *et al* reported the most common components of the essential oil of *A. alba* from the territory of Gradište - Sićevačka Gorge to be camphor (33.2-36.8%), 1,8-cineole (15.2-21.1%), artemisia ketone (6.0-24.2%), artemisia alcohol (2.6-3.2%) and borneol (3.0-3.6%). However, they found higher amounts of methyl eugenol (3.6%) in comparison to the results of our study (0.23%) [19]. In addition, essential oil obtained from *A. alba* herb from the territory of Kokin Brod was shown to contain camphor (31.0%), 1,8-cineole (14.4%), artemisia ketone (8.7%) and borneol (3.7%). This essential oil contained thujone (10.5%), while this monoterpenoid was not identified in our research [34]. The presence of this compound has also been shown in small amounts in the essential oils of *A. alba* herb from different locations in Italy [35]. Likewise, essential oils of other *Artemisia* species has been shown to posses high contents of 1,8-cineole (21.5-27.6%) and camphor (15.9-37.3%) [9].

In contrast to the essential oil, chemical composition of *A. alba* herb extracts has been poorly described in the literature. Our investigation revealed qualitative and quantitative contexture of both volatile and non-volatile fractions of the ethanolic extract. Volatile fraction of the investigated extract has shown to be different in comparison to the essential oil. Namely, the dominant compounds found in the essential oil (camphor, 1,8-cineole and artemisia ketone) were present in the extract in much lower quantities. The results of chemical composition of non-volatile fraction of the tested extracts show that the dominant compounds are phenolic acids and flavonol derivatives. Kaempferol was also a major flavonol in extracts of *Artemisia* species studied by Carvalho and associates [17].

The absence of α-thujone and β-thujone in the essential oil and extract is a very important fact, while it allows these isolates to safely be added to a variety of products. Thujone is one of the most toxic compounds which can cause a number of disorders in the body, when used for a long time, even in small quantities. Thus, its concentration in products is strictly limited [36].

Antioxidants play an important role in the inhibition of free radicals, thus providing protection of the human body against infectious and degenerative diseases. The antioxidant activity of the tested extract is higher compared to the essential oil, which can be correlated to a higher content of total phenolics and flavonoids. Chlorogenic acid, apigenin and luteolin, kaempferol flavonols, quercetin, and their heterosides are very important antioxidant agents [17,37]. Rutin exhibited strong DPPH radical scavenging activity [38]. Low antioxidant capacity of the essential oil, demonstrated in our investigation, is in correlation with the results of other researches [9,39].

The rapidly increasing incidence of bacterial resistance to antimicrobial agents has become a serious problem worldwide. The emergence of multidrug-resistant organisms (methicillin-resistant *Staphylococcus aureus,* glycopeptide-resistant *Enterococci,* aminoglycoside resistant *Klebsiella* spp. and *Pseudomonas aeruginosa*) represents a problem for both the treatment of patients and control of infection. Besides conventional antimicrobial agents, numerous studies reported antibacterial activity of various plant extracts and their synthesized counterparts. Antimicrobial activity of plants is mainly caused by small molecules like terpenoids, flavonoids and polyphenols.

Our results revealed that the tested isolates of *A. alba* exerted significant antimicrobial activity against all tested microorganisms. These results are consistent with their chemical composition. Namely, previous investigations have shown that oxygenated monoterpenes, such as 1,8-cineole, camphor, terpinen-4-ol, linalool, α-terpineol and borneol exhibit strong antimicrobial activity [13,39]. Also, significant antimicrobial activity has been reported for phenolic compounds, identified in the investigated *A. alba* herb extract [37].

5. Conclusions

Investigated extract of *A. alba* herb exhibited stronger antioxidant activity than the essential oil as well as better antimicrobial activity on a larger number of tested microorganisms. On the basis of these findings, while bearing in mind traditional use of plant species belonging to the genus *Artemisia*, as bitter aromatic drugs, and their beneficial effects in the treatment of

digestive disorders, the obtained results provide scientific footing and momentum for further investigation of their rational therapeutic use. The absence of α- and β-thujone in extract is important information for the safe usage in liquid pharmaceutical bitter aromatic preparations and/or bitter liqueurs. In addition, from technological point of view, its incorporation into such products is easier compared to the essential oil.

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