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Reishi (*Ganoderma lucidum*) tincture enriched with propolis or green tea from Serbia: UHPLC-DAD–ESI-MS characterization, mineral content and antibacterial activity

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Abstract: The objective of the present study was to evaluate the differences in chemical composition and mineral content of pure Reishi (*Ganoderma lucidum*) tincture with those enriched with propolis or green tea. The chemical composition and mineral content were analyzed by UHPLC-DAD–ESI-MS and ICP-OES methods, respectively. Among all samples, Reishi/propolis tincture contained a significant amount of polyphenols, with the dominant contribution of flavonoids and phenolic acids. Regarding mineral content, P, K and Na were the most abundant in all tinctures. Reishi/propolis tincture also demonstrated the best antibacterial activity, with *Proteus vulgaris* and *Pseudomonas aeruginosa* as the most sensitive bacteria.

Keywords: antibacterial activity; green tea; ICP-OES; propolis; reishi; UHPLC––DAD-ESI-MS.

INTRODUCTION

Reishi or Lingzhi (*Ganoderma lucidum*) is one of the most popular and highly regarded medicinal fungi, demonstrating various biological and pharmacological activities, *e.g.*, anti-tumor, antimicrobial, antioxidative, anti-inflammatory, anti-hyperglycemic, anti-hyperlipidemic, anti-tyrosinase and antimutagenic, as well as cardio-, neuro-, hepato- and renal-protective, *etc.*[1,2] Commercial *G. lucidum* products are available as powders, dietary supplements, and tea obtained from its different parts, including mycelia, spores and fruit bodies. The main component of mushrooms is water (90 mass %), while proteins, fats, carbohydrates, ash, minerals and vitamins are the remaining 10 mass % of the total content.[2,3] The fruit body of this

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fungus is a rich source of bioactive components, such as polysaccharides, triterpenes, polyphenols, vitamins and different types of secondary metabolites (phenolic compounds, polyketides, terpenes, steroids and phenols), including more than 140 different lanostane-type triterpenoids.[1–3] Such a versatile chemical composition explains a wide range of its pharmaceutical properties.

Combining pure Reishi tincture with other natural and biologically active extracts can improve its biological and pharmacological activities. Propolis, a resinous substance collected by bees from different plants, is another natural product considered one of the best general panaceas since ancient times.[4] It is a complex mixture, and its chemical composition is qualitatively and quantitatively variable, depending on the bee species, geographical areas and botanical source.[5,6] Among natural compounds found in propolis, polyphenols are the most important, mainly phenolic acids and flavonoids.[4–6] These compounds are responsible for its numerous activities, such as antimicrobial, antioxidant, anti-inflammatory, antiproliferative, antihypertensive, spasmolytic, hepatoprotective, immune-stimulant, *etc*.[4–6] Propolis can be found in various cosmetics, beverages, food products, and dietary supplements. Green tea, obtained from the leaves of *Camellia sinensis* L., is a widely consumed beverage worldwide. It is rich in polyphenols (particularly catechins), while its extract contains lower amounts of other compounds, such as flavonoids and phenolic acids.[7]

Thus, the aims of the current study were to determine and compare the chemical and mineral composition of pure Reishi tincture with those enriched with propolis or green tea from Serbia, as well as to evaluate their antimicrobial activity against eight bacteria. The chemical composition and mineral content were analyzed by UHPLC-DAD–ESI-MS and ICP-OES methods, respectively. These results could promote further development of the Reishi production industry in Serbia and be useful for the food, pharmaceutical, and cosmetic industries.

EXPERIMENTAL

Material

Commercial ethanolic extracts obtained by triple extraction of pure Reishi (35 %), Reishi/ /propolis (35 %), and Reishi/green tea (35 vol. %) were analyzed. A total of 100 ml of each tincture was evaporated by rotary evaporator at 40 °C to dryness and dried at 50 °C until reaching constant weight. The total solid matter was 4 $mg \cdot mL^{-1}$ of tincture.

Chemicals and reagents

Ethanol, 96 % (Centrochem, Zemun, Serbia), and quercetin, rutin, and apigenin standards (Sigma-Aldrich, St. Louis, USA) were used. Distilled water and aqueous NaCl solution (0.9 %) were provided from Zorka Pharma d.o.o. (Šabac, Serbia). Dimethylsulfoxide (DMSO, ≥ 99 %) was purchased from Sigma–Aldrich (Munich, Germany). Solvents used in the UHPLC experiments were of HPLC or LC/MS grade. Concentrated nitric acid (65 %, Merck) and distilled water (HPLC grade, Fisher Chemical) were used for ICP-OES analysis.

UHPLC-DAD–ESI-MS analysis

The chromatographic separation was performed on a Hypersil Gold C18 column (50 mm×2.1 mm, 1.9 μm) at 25 °C using a Dionex Ultimate 3000 UHPLC+ system equipped with a diode array (DAD) detector and LCQ fleet ion trap mass spectrometer (Thermo Fisher Scientific, USA). A mobile phase consisted of two solvents, 0.1 vol. % formic acid in water and methanol, with a gradient program at 0.25 mL·min^{-1} flow rate.[8] The injection volume was 1 μl. Absorption UV/Vis spectra were recorded on a DAD-detector with a total spectral range between 200 and 800 nm. The mass spectrometric analysis was performed using a 3D-ion trap with electrospray ionization (ESI) in negative ion mode. The ESI source parameters were as follows: source voltage 4.5 kV, capillary voltage 41 V, tube lens voltage 95 V, capillary temperature 350 °C, nitrogen sheath and auxiliary gas flow 32 and 8 arbitrary units. MS spectra were acquired by full range acquisition of *m*/*z* 130–1300, with a tandem mass spectrometry analysis performed by a data-dependent scan – the collision-induced dissociation (CID) of detected molecular ion peaks ([M–H]$^-$) with normalized collision energy set at 25 eV. The ESI source parameters for positive ion mode also used for detection of pure Reishi tincture were source voltage 5.0 kV, capillary voltage 49 V, tube lens voltage 100 V, capillary temperature 350 °C, and 32 and 8 units for the auxiliary and sheath gas flow, respectively, in the full range, 130––1200, and data-dependent scan with CID set at 25 eV of detected $[M+H]^+$, or cationized $[M+Na]^+$ molecular ion peaks. Xcalibur software (version 2.1) was used for instrument control, data acquisition and data analysis.

ICP-OES analysis

The quantitative analysis was carried out using inductively coupled plasma-optical emission spectrometry (ICP-OES, Arcos FHE12, Spectro, Germany), following the manufacturer's guidelines. All samples from the solid (Reishi, propolis and green tea powders) and liquid (tinctures) materials were prepared through a wet digestion procedure with concentrated nitric acid, as detailed in the literature.[9,10] Argon 5.0 (99.999 %) purity served as the carrier gas. In addition to the analytical sample, calibration standards were prepared beforehand. The calibration solution was created using Multistandard IV – a multi-elemental standard solution from Merck, which includes Ag, B, Cd, Co, Cr, Cu, Fe, Li, Mn, Na, Ni, Pb, Tl and Zn at concentrations of 1000 ppm. Parameters of the calibration lines for the determined elements are presented in the Supplementary material to this paper (Table S-I).

Antimicrobial activity

The antimicrobial activity was evaluated using the following microorganisms: *Staphylococcus aureus* (ATCC 25923), *Psudomonas aeruginosa* (ATTC 27853), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 700603), *Listeria monocytogenes* (ATCC 19166), *Proteus vulgaris* (ATCC 8427), *Bacillus cereus* (ATCC11778) and *Bacillus luteus in haus* strain. Microorganisms were from the Microbiology Laboratory, Faculty of Technology, Leskovac. Mediums used for the growth of the microorganisms were Antibiotic agar No. 1 for microbiology (Merck, Darmstadt, Germany) and nutrient agar (Torlak, Belgrade, Serbia) for bacterial growth. The agar disc diffusion method was used for testing the antibacterial activity.[11] The mediums were sterilized in an autoclave at 121 °C under 110 kPa for 25 min. The suspension was prepared with overnight culture and adjusted to 0.5 McFarland standard. The prepared suspension contained 1×10^8–2×10^8 colony-forming unit (CFU)·mL^{-1}. The inoculum of 0.1 mL of suspension was added to 10 mL of medium and poured into the Petri dishes. For screening, sterilized filter paper disks (9 mm in diameter, Schleicher & Schuell) were placed on the surface of inoculated mediums and impregnated with 50 μL of the extract sample (evaporated ethanolic extract

dissolved in DMSO, concentration 100 mg·mL^{-1}). Pure DMSO was used as a negative control. Plates were incubated for 24 h at 37 °C and results were expressed as the diameter of inhibition zones (IZ in mm). The presence of the IZ indicated the activity of the tested samples against the tested microorganisms. The following commercial standardized discs with predetermined amounts of antibiotics were used as positive controls: ciprofloxacin (5 μg), imipenem (10 μg), penicillin (6 μg, 10 IU), trimethoprim/sulfamethoxazole (1.25/23.75 μg), clindamycin (2 μg), ceftriaxone (30 μg), gentamicin (10 μg), amoxicillin + clavulanate (20/10 μg) and cefoxitin (30 μg), all from BD Sensi-Disc, GmbH, Heidelberg. All experiments were performed in triplicate.

Statistical analysis

All results were reported as mean value ± standard deviation from three measurements. Statistical comparisons were carried out by one-way ANOVA (analysis of variance) followed by Tukey's multiple comparison test by software SPSS 23.0 (IBM, USA). Differences are reflected as significant at $p < 0.05$.

RESULTS AND DISCUSSION

UHPLC-DAD–ESI-MS analysis

The typical UHPLC-DAD chromatograms recorded from DAD-signal at λ = = 320 nm are shown in Fig. 1a–c.

The list of all compounds detected with their MS/UV/Vis spectral data is shown in Tables S-II–S-IV of the Supplementary material. For some detected compounds, the absorption and MS/MS spectra are shown in Supplementary matrial (Figs. S-1 and S-2). Mass spectra and fragmentation patterns, the order of elution from the chromatographic system, together with literature data,[13–28] allowed the identification of some detected compounds.

Basic physiologically active components in *G. lucidum* are polysaccharides, peptidoglycans and triterpenes.[1–3] Among 13 registered DAD-signals in pure Reishi tincture (Fig. 1a, Table S-II), signal 10 was identified as 12-acetoxyganoderic acid F, according to $[M–H]^–$, *m*/*z* 569, while ganoderic acid G type A ($[M–H]^–$, *m*/*z* 531 in negative mode and $[M+Na]^+$, *m*/*z* 555 in positive mode) was assigned to peak 12.[16] Those ganoderic acids (related to triterpenoids) are the most representative components in *G. lucidum*, responsible for different biological activities. An appropriate MS/MS-fragmentation pattern in positive mode for the same compound (Fig. S-1a) confirmed the suggested compound, with cationized adducts at *m/z* 555 $[M+Na]^+$ and corresponding fragment ions. The corresponding UV/Vis spectrum (Fig. S-1a) of the ganoderic acid G type A exhibits an absorption maximum at 258 nm.[16] The triterpenoid, often present in *G. lucidum*, lucidone A, was tentatively identified by confirming with Yan *et al*.[15]

The dominant class of compounds in both Reishi/propolis and Reishi/green tea tinctures are flavonoids. Rutin (Fig. 1b, Table S-III; peak No. 11), quercetin (Fig. 1c, Table S-IV; peak No. 9), and apigenin (Fig. 1c, Table S-IV; peak No. 14) were identified by their standard methanolic solutions. Hydroxybenzoic acid, caffeic acid, *p*-coumaric acid, caffeic acid derivate and dihydroquercetin (Fig. 1c,

Table S-III; peaks No. 1, 2, 3, 4 and 5, respectively) were identified by using MassBank Record.[19] For qualitative analysis of all detected signals, mass spectrometry combined with collision induced dissociation of molecular ions in data-dependent mode MS/MS was used, together with a comparison with the corresponding literature (Tables S-III and S-IV).

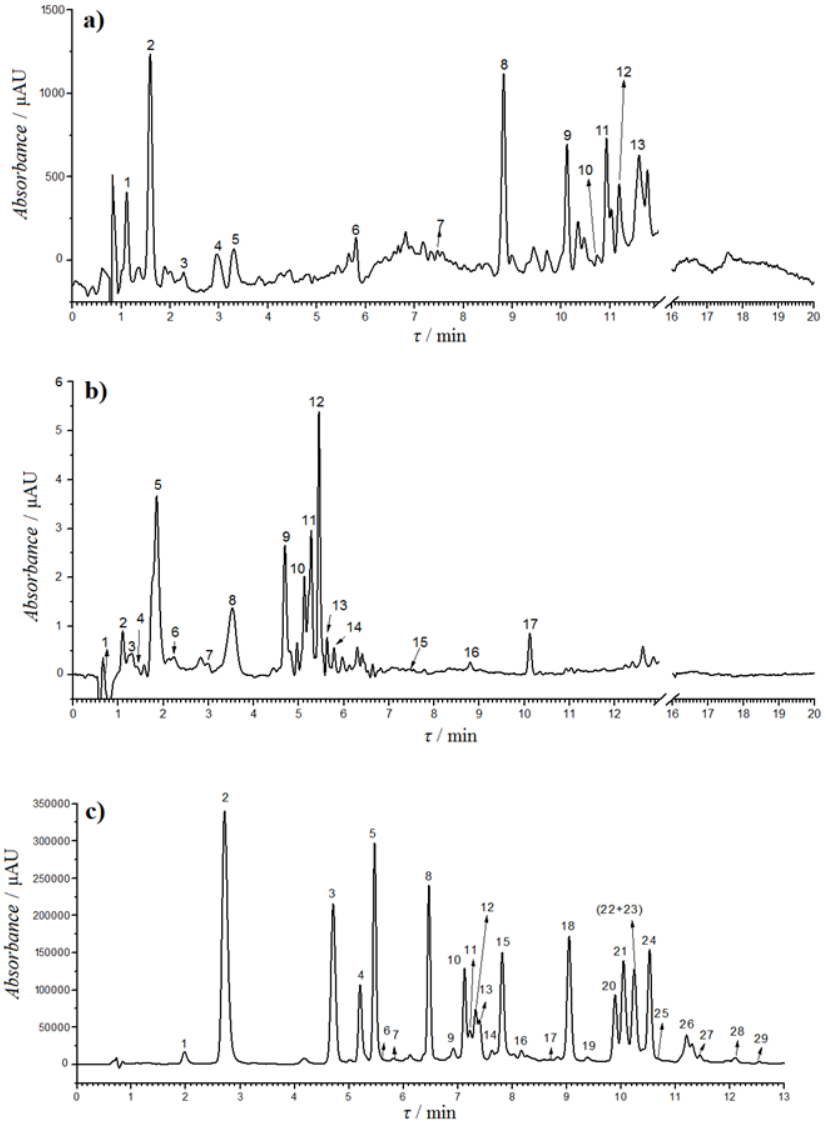

Fig. 1. UHPLC-DAD chromatograms of all tinctures (a – pure Reishi, b – Reishi/green tea and c – Reishi/propolis) at DAD signal λ_{det} = 300 nm. The corresponding peaks assignments (in the chromatograms labeled by numbers) are listed in Tables S-I–S-III, respectively.

Reishi/green tea tincture is rich in catechin, epicatechin, gallocatechin, epigallocatehin, and flavan-3-ols, characteristic of green tea. Gallocatechin is detected as signal No. 2 in the UHPLC-DAD chromatogram (Fig. 1b, Table S-III) and confirmed by the MS/MS-fragmentation pattern in negative mode and correspondding fragment ions (Fig. 2b). The UV/Vis spectrum of gallocatechin (Fig. S-1b) exhibits an absorption maximum at 287 nm.[18,29] Reishi/green tea tincture also contains flavonols: kaempferol 3-*O*-rutinoside, kaempferol-*O*-glycoside, quercetin-glycoside and its quercetin-3-*O*-rutinoside derivative rutin (Fig. 1b, Table S-III; peaks No. 13, 14, 12 and 11, respectively) and flavones such as apigenin-glucosides: apigenin 6 (or 8)-C-xyloside-8(or 6)-C-glucoside (known as vicenin 1, IUPAC name 5,7--dihydroxy-2-(4-hydroxyphenyl)-8-[(2*S*,3*R*,4*R*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]-6-[(2*S*,3*R*,4*S*,5*R*)-3,4,5-trihydroxyoxan-2-yl]chromen-4-one) and apigenin 6-C-glucosyl 8-C-(2"-*O*-dihydroferuloyl)-glucoside (IUPAC name 5,7-dihydroxy-2-(4-hydroxyphenyl)-6-[(2*S*,3*R*,4*R*,5*S*,6*R*)-3, 5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]-8-{[2*R*,3*R*,4*S*,5*S*,6*R*)-2-[3-(4-hydroxy-3-methoxyphenyl)propanoyl]oxy-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]}chromen-4-one) (Fig. 1b, Table S-III; peaks No. 9 and 10, respectively). MS/MS fragmentation pattern in a negative mode for vicenin 1 (Fig. S-1c) presents molecular ions at *m*/*z* 563, 443, 383 and 353. These neutral losses are characteristics of glucosyl and pentosyl residues. The fragmentation signals at *m*/*z* 383 and 353 are also typical for di-*C*--glycosylflavones. UV/Vis spectra of vicenin 1 (Fig. S-1c) exhibit two absorption maxima at 272 and 334 nm, which is in accordance with the literature.[20] Quercetin--glycoside (Fig. 1b, Table S-III; peak No. 12) is detected as quercetin-7-*O*-glycoside, since the fragment ion at *m*/*z* 301 is rather abundant in 7-*O*-glycoside than in 3-*O*-glycoside (Fig. S-1d). Moreover, UV/Vis absorption maxima at 271 and 349 nm are in the range for this class of flavonols.[21]

Reishi/propolis tincture is rich in phenolic acids. Caffeic acid was detected as peak No. 2 in the UHPLC-DAD chromatogram (Fig. 1c, Table S-IV), while the corresponding UV/Vis spectrum (Fig. S-2a) contains two absorption maxima at 290 and 323 nm. An appropriate MS/MS-fragmentation pattern in negative mode (Fig. S-2a) confirmed the suggested compound with molecular ion at *m*/*z* 179 $[M–H]^-$ and corresponding fragment ion at *m*/*z* 135.[19,23] Caffeic acid derivatives were detected by peaks No. 4, 20, 21 and 22 (Fig. 1c, Table S-IV).[19,23–25,28] Reishi/propolis tincture also contains quercetin and its derivatives dihydroquercetin or taxifolin, quercetin-*O*- rhamnoside, quercetin-3-methyl-ether and quercetin-3-dimethyl-ether (Fig. 1c, Table S-IV; peaks No. 9, 5, 7, 11 and 19) with molecular ion peaks found at *m*/*z* 301, 303, 447, 300 and 329, respectively. The corresponding UV/Vis spectrum of taxifolin (Fig. S-2b) contains two absorption maxima at 298 and 323 nm, while an appropriate MS/MS-fragmentation pattern in negative mode (Fig. S-2b) confirmed the suggested compound.[19,25] Genistin, a well-known isoflavone, is identified as a peak No. 8 ($[M–H]^-$, *m*/*z* 431) (Fig. 1c,

Table S-IV). Corresponding UV/Vis spectrum (Fig. S-2c) contains two absorption maxima at 290 and 322 nm, typical for this compound. The product ion mass spectrum of $[M-H]^-$ (Fig. S-2c) shows neutral losses of the glycan residue (162 Da) and of the glycan residue $+H^\bullet$ (163 Da) by rearrangement and cleavage, respectively, which is expected since, structurally, genistin represents genistein 7--*O*-*β*-D-glucoside.[19,25] Reishi/propolis tincture is also rich in dihydroflavonol pinobanksin, its 5-methyl-ether derivative and 3-*O*-derivatives (acetate, butyrate, propionate and pentanoate), as well as methoxy-chrysin, luteolin-methyl-ether, galangin-5-methyl-ether and isorhamnetin-3-O-rutinoside.[24,26,28] The tincture also contains flavanone pinocembrin (Fig. 1c, Table S-IV; peak No. 23), a natural precursor of pinobanksin. The corresponding UV/Vis spectrum (absorption maxima at 291 and 356 nm) and MS/MS-fragmentation pattern (Fig. S-2d) confirmed its presence.[28] Peaks No. 12, 13, 18 and 26 were unidentified derivatives of phenolic acids and flavonoids.

Mineral content

ICP-OES analysis results are presented in Table I.

TABLE I. Concentrations of macro- and microelement in samples; column values (different capital letters) and row values (different small letters) are significantly different ($p < 0.05$) by Tukey's multiple range test

Mineral	Solid material, c / μg·g^{-1}			Tincture, c / mg·L^{-1}		
	Reishi	Green tea	Propolis	Reishi	Reishi/green tea	Reishi/propolis
Ag	6.45 ^{La}	0 ^{Kb}	0 ^{Nb}	0 ^{Kb}	0 ^{Ob}	0 ^{Mb}
B	6.05 ^{Mb}	3.54 ^{Gc}	31.77 ^{Ha}	0 ^{Ke}	3.28 ^{Ed}	0 ^{Me}
Ca	0 ^{Oc}	112.1 ^{Bb}	760.43 ^{Da}	0 ^{Kc}	0 ^{Oc}	0 ^{Mc}
Cd	0 ^{Oc}	6.8 ^{Fa}	0 ^{Nc}	0 ^{Kc}	0 ^{Oc}	0.025 ^{Lb}
Co	0 ^{Ob}	0.03 ^{Ka}	0 ^{Nb}	0 ^{Kb}	0 ^{Ob}	0 ^{Mb}
Cr	44.76 ^{Ja}	0.45 ^{J,Kb}	0 ^{Nc}	0 ^{Kc}	0 ^{Oc}	0 ^{Mc}
Cu	77.02 ^{Fa}	2.45 ^{Hc}	8.49 ^{Lb}	0.15 ^{If}	0.83 ^{Jd}	0.4 ^{Ie}
Fe	1071.78 ^{Ca}	2.2 ^{Hc}	26.43 ^{Ib}	0 ^{Ke}	0.43 ^{Ld}	0 ^{Me}
K	2.02 ^{Nf}	11 ^{Ee}	2659.07 ^{Ba}	89.78 ^{Bb}	49.33 ^{Bc}	26.25 ^{Cd}
Li	174.6 ^{Ea}	2.2 ^{Hb}	1.21 ^{Mc}	0.1 ^{Je}	0.13 ^{Md}	0.1 ^{Ke}
Mg	0 ^{Oe}	320 ^{Ab}	383.12 ^{Ea}	4.9 ^{Ec}	2.95 ^{Gd}	2.05 ^{Fd}
Mn	739.11 ^{Da}	0.5 ^{J,Kc}	15.28 ^{Jb}	0 ^{Ke}	0.075 Nd	0 ^{Me}
Na	0 ^{Of}	93 ^{Cb}	318.14 ^{Fa}	35.6 ^{Cd}	48.23 ^{Cc}	34.48 ^{Ae}
Ni	13.31 ^{Ka}	2.35 ^{Hb}	0 ^{Nc}	0 ^{Kc}	0 ^{Oc}	0 ^{Mc}
Pb	45.16 ^{Ia}	1.15 ^{Ic}	0 ^{Ne}	0 ^{Ke}	1.45 ^{Ib}	0.25 ^{Jd}
Sr	0 ^{Oe}	0 ^{Ke}	11.4 ^{Ka}	0.53 ^{Hc}	0.73 ^{Kb}	0.5 ^{Hd}
Tl	47.18 ^{Ha}	0.53 ^{J,Kb}	0 ^{Nc}	0 ^{Kc}	0 ^{Oc}	0 ^{Mc}
Zn	48.79 ^{Ga}	0.3 ^{Kf}	36.13 ^{Gb}	2.38 ^{Gc}	2.35 ^{Hd}	1.7 ^{Ge}
Si	5657.26 ^{Aa}	2.25 ^{Hf}	35.89 ^{Gb}	3.45 ^{Fd}	3.08 ^{Fe}	3.85 ^{Ec}
P	1594.76 ^{Bb}	0.88 ^{I,Jf}	8692.05 ^{Aa}	238.48 ^{Ac}	117.58 ^{Ad}	31.15 ^{Be}
S	0 ^{Of}	29.25 ^{Db}	1244.91 ^{Ca}	26.2 ^{Dc}	21.88 ^{Dd}	6.53 ^{Be}

The difference in mineral content between crude samples and tinctures is evident, not only in a qualitative but also in a quantitative manner. A total of 21 elements was determined. Tested Reishi powder is notable for its high content of Si (5657.26 $\mu g \cdot g^{-1}$), P (1594.76 $\mu g \cdot g^{-1}$), Fe (1071.78 $\mu g \cdot g^{-1}$) and Mn (739.11 $\mu g \cdot g^{-1}$), while propolis was rich in P (8692.05 $\mu g \cdot g^{-1}$), K (2659.07 $\mu g \cdot g^{-1}$), S (1244.91 $\mu g \cdot g^{-1}$) and Ca (760.43 $\mu g \cdot g^{-1}$), followed by Mg (383.12 $\mu g \cdot g^{-1}$) and Na (318.14 $\mu g\ g^{-1}$). Among all crude samples, green tea powder contained the least amount of minerals, with Mg as the most abundant element (320 $\mu g \cdot g^{-1}$), followed by significantly lower quantities of Ca (112.1 $\mu g \cdot g^{-1}$), Na (93 $\mu g \cdot g^{-1}$) and S (29.25 $\mu g \cdot g^{-1}$). Although the mineral content of plants depends upon soil structure, climatic conditions, and plant species, the basic elements are more or less present in the same species. According to literature, Reishi contains minerals like Cu, Zn, Se, K, Ca, P, Mg, and Fe.[30] Propolis possesses Ca, K, Al, Cu, Mg, Fe and Zn in the highest amounts.[31] The most abundant minerals found in green tea are P, K, Ca, Mg, Fe, Mn, Al, S, Si, followed by Zn, Cu and F.[32] In all Reishi tinctures, the most common minerals are P, K and Na, which are very important macro-elements and have a large impact on the human body. P plays a crucial and vital part in the creation of bones, teeth, nucleic acids, phosphoproteins, growth and the control of different repair processes. Its role in the conversion of food into energy, the metabolism of lipids and carbohydrates, lessening arthritis pain, and restoring proper kidney and cardiac function is also essential.[33] As two essential macroelements, K and Na are important to maintain body cellular homeostasis and metabolism, since most metabolic processes are dependent on or affected by these electrolytes. Also, these electrolytes maintain normal pH levels in extracellular fluid, regulate osmotic pressure, act as cofactors for many enzymes, and are involved in oxidation–reduction reactions.[34] Compared to crude samples, in all Reishi extracts, toxic elements, such as Cd and Pb, were not found or were present in insignificant amounts.

Antimicrobial activity

The results of the disc diffusion testing are presented in Table II. Both DMSO and sterile water negative controls showed no inhibition effects. The inhibition zones reveal different susceptibility of different bacteria to all tinctures.

None of the tinctures was antibacterial against *K. pneumonia*. While pure green tea tincture failed to express any antibacterial activity, both the pure Reishi and propolis tinctures demonstrated similar potential against *P. aeruginosa*, *E. coli* and *P. vulgaris*. However, propolis tincture was more active against *S. aureus* and was the only pure tincture deemed as antibacterial against *L. monocytogenes*, *B. cereus* and *B. luteus*. The antibacterial potential of different Reishi tinctures was reported in earlier studies.[35,36]

TABLE II. Values of zones of bacterial growth inhibition (mm); column values (different capital letters) and row values (different small letters) are significantly different ($p < 0.05$) by

Tukey's multiple range test; NI – no inhibition; NT – not tested. *According to the CLSI protocol microorganisms can be consider resistant if the inhibition zone iz lower than 14 mm, intermediate or susceptible-dose dependent for the zone 15–19 mm and susceptible for the zone bigger than 20 mm; bacteria: SA – *S. aureus*; PA – *P. aeruginosa*; EC – *E. coli*; KP – *K. pneumoniae;* LM – *L. monocytogenes*; BC – *B. cereus*; BL – *B. luteus*; PV – *P. vulgaris*; tincture: R – Reishi; GT – green tea; P – propolis; antibiotics: CPF – ciprofloxacin; TMF/SMT – trimethoprim/sulfamethoxazole; PCL – penicillin; CDM – clindamycin; CTX – ceftriaxone; IPN – imipenem; GTC – gentamicin; AMX/CVT – amoxicillin/ clavulanate; CFX – cefoxitin

Inhibitor	SA	PA	EC	KP	LM	BC	BL	PV
R	$9^{*}\pm0.6^{Jc}$	12 ± 0.5^{Hb}	10 ± 0.5^{Gc}	NI^{Gd}	NI^{Hd}	NI^{Fd}	NI^{Id}	15 ± 0.5^{Fa}
GT	NI^{Ka}	NI^{Ia}	NI^{Ha}	NI^{Ga}	NI^{Ha}	NI^{Fa}	NI^{Ia}	NI^{Ha}
P	17 ± 0.6^{Ia}	$13\pm0.6^{Hb,c}$	12 ± 0.6^{Fc}	NI^{Gd}	16 ± 0.5^{Ga}	14 ± 0.5^{Eb}	12 ± 0.6^{Hc}	$13\pm0.6^{Gb,c}$
R/GT	11 ± 0.6^{Jc}	15 ± 0.5^{Ga}	$12\pm1^{Fb,c}$	NI^{Gd}	NI^{Hd}	13 ± 0.6^{Eb}	NI^{Id}	$16\pm0.5^{E,Fa}$
R/P	21 ± 0.6^{Ga}	17 ± 0.4^{Fb}	15 ± 0.6^{Ec}	NI^{Gd}	20 ± 0.5^{Ea}	17 ± 0.2^{Db}	15 ± 0.3^{Gc}	17 ± 0.5^{Eb}
CPF	$26\pm0.6^{D,Ee}$	40 ± 0.5^{Aa}	40 ± 0.6^{Aa}	31 ± 0.8^{Ac}	36 ± 0.6^{Bb}	$30\pm1^{Ac,d}$	$28\pm1^{Dd,e}$	26 ± 0.6^{Ce}
TMF/ SMT	NT^{Kb}	NI^{Ib}	NT^{Hb}	NT^{Gb}	NT^{Hb}	NT^{Fb}	21 ± 0.6^{Fa}	NT^{Hb}
PCL	32 ± 1^{Ba}	$16\pm0.5^{F,Gc}$	NT^{He}	11 ± 1^{Fd}	16 ± 0.6^{Gc}	13 ± 0.5^{Ed}	29 ± 1^{Db}	$31\pm1^{Ba,b}$
CDM	$22\pm0.8^{F,Ga}$	NI^{Ic}	NT^{Hc}	NT^{Gc}	18 ± 0.5^{Fb}	NT^{Fc}	NT^{Ic}	NT^{Hc}
CTX	$28\pm1^{C,Db}$	32 ± 0.5^{Ba}	32 ± 0.6^{Ba}	$22\pm0.5^{C,Dc}$	27 ± 0.3^{Cb}	21 ± 1^{Cc}	14 ± 1^{Gc}	31 ± 0.3^{Ba}
IPN	40 ± 1^{Ab}	22 ± 1^{Dd}	27 ± 0.6^{Cc}	26 ± 0.6^{Bc}	42 ± 1^{Ab}	26 ± 0.6^{Bc}	46 ± 0.6^{Aa}	42 ± 0.5^{Ab}
GTC	$24\pm0.8^{E,Fa,b}$	$20\pm0.5^{Ec,d}$	$22\pm1^{Db,c}$	$20\pm1^{Ec,d}$	25 ± 0.6^{Da}	$21\pm0.6^{Cc,d}$	26 ± 0.6^{Ea}	19 ± 0.5^{Dd}
AMX/ CVT	$30\pm0.6^{B,Cb}$	26 ± 0.6^{Cc}	12 ± 1^{Fe}	$21\pm1^{D,Ed}$	$26\pm0.6^{C,Dc}$	21 ± 1.05^{Cd}	44 ± 0.6^{Ba}	42 ± 1^{Aa}
CFX	$28\pm0.6^{C,Db,c}$	26 ± 1^{Cc}	$22\pm0.5^{Dd,e}$	23 ± 0.5^{Cd}	20 ± 1^{Ee}	$22\pm0.5^{Cd,e}$	40 ± 0.5^{Ca}	30 ± 1^{Bb}

Compared to the pure Reishi extract, the Reishi/green tea tincture exhibited improved antibacterial effect, while Reishi/propolis tincture showed the highest activity against all tested strains, especially *S. aureus* and *L. monocytogenes*. Somewhat improved antibacterial activity of Reishi/green tea tincture compared to the pure Reishi and pure green tea tinctures can only be explained by the possible synergistic effect of the components present in Reishi and green tea. Specifically, the main catechins present in green tea (epicatechin, epicatechin-3-gallate, epigallocatechin and epigallocatechin-3-gallate) are known antimicrobials;[37] these catechins are detected in prepared Reishi/green tea tincture.

Interestingly, the addition of propolis in the Reishi tincture enabled it to express activity against *L. monocytogenes*, *B. luteus* and *B. cereus*. Previous studies confirmed the antibacterial potential propolis tinctures from Serbia against *L. monocytogenes*, *S. aureus* and *E. coli*.[38–40] Although the chemical composition of propolis is quite complex, its source causes only minor variations regarding its antimicrobial activity.[40] Antimicrobial activity of propolis was associated with the presence of phenolic compounds, such as caffeic acid, quercetin, luteolin, apigenin,

p-coumaric acid, kaempferol and pinobanksin.[39] Since these phenolic components are also present in Reishi/propolis tincture, its improvement in antibacterial capacity is explained by both the presence of various phenolic compounds originating from Reishi and propolis, as well as their synergistic effect.

Although the Reishi/propolis tincture exhibits moderate antibacterial activity compared to some commercial options, its use may still be justified due to the multiple other potential health benefits associated with its constituents. Previously reported immunomodulatory, anti-inflammatory and antioxidant properties of both Reishi[1–3] and propolis[4–6] could contribute to overall wellness.

CONCLUSION

The chemical composition and mineral content of pure Reishi tincture and those enriched with propolis or green tea were compared. Among all samples, Reishi/propolis tincture contained a significant amount of polyphenols, with the dominant contribution of flavonoids and phenolic acids. Regarding mineral content, P, K and Na were the most abundant in all tinctures. Reishi/propolis tincture also exhibited the best antibacterial activity compared to the other extracts. Based on reported chemical compositions and antimicrobial activities, these Reishi-based tinctures have promising prospects as food and health supplements. The high levels of polyphenols, flavonoids, phenolic acids and essential minerals may contribute to antioxidant, immunomodulatory, and general wellness-promoting effects. Enrichment with propolis or green tea further enhances the bioactive profile, potentially supporting cardiovascular, metabolic, and immune health. Therefore, even if the antibacterial activity is moderate compared to commercial products, the combined health benefits and nutritional value make these tinctures valuable candidates for dietary supplementation. Further studies on bioavailability, dosage and clinical effects would help to fully realize their potential in human health applications.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/13428, or from the corresponding author on request.

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ИЗВОД

ТИНКТУРА РЕИШИЈА (*Ganoderma lucidum*) ОБОГАЋЕНА ПРОПОЛИСОМ ИЛИ ЗЕЛЕНИМ ЧАЈЕМ ИЗ СРБИЈЕ: UHPLC-DAD–ESI-MS КАРАКТЕРИЗАЦИЈА, МИНЕРАЛНИ САСТАВ И АНТИБАКТЕРИЈСКА АКТИВНОСТ

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Циљ овог рада био је процена разлика у хемијском саставу и минералном садржају чисте реиши (*Ganoderma lucidum*) тинктуре са тинктурама обогаћеним прополисом или зеленим чајем. Хемијски састав и садржај минерала су анализирани применом течне хроматографије веома високих перформанси у комбинацији са детектором умрежених фотодиода и електроспреј јонизација-масеном спектрометријом (UHPLC-DAD–ESI-MS) и оптичком емисионом спектрометријом са индуктивно спрегнутом плазмом (ICP-OES), редом. Међу овим узорцима, реиши/прополис тинктура садржи значајну количину полифенола са доминантним уделом флавоноида и фенолних киселина. У погледу минералног састава, P, K и Na су највише присутни у свим тинктурама. Реиши/прополис тинктура је показала и најбољу антибактеријску активност, при чему су *P. vulgaris* и *P. aeruginosa* биле најосетљивије бактерије.

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