ORIGINAL ARTICLE

PHYTOCHEMICAL ANALYSIS, ANTIMICROBIAL AND ANTIOXIDANT EFFECT OF SOME GEMMOTHERAPIC REMEDIES USED IN RESPIRATORY DISEASES

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Manuscript received: September 2015

Abstract

The major objective of this study was the investigation of the antioxidant and antimicrobial potential of some gemmotherapic remedies obtained from several plants: *Betula pubescens* (BP), *Ribes nigrum* (RN), *Rosa canina* (RC), *Viburnum lantana* (VL) and *Carpinus betulus* (CB) often combined in therapeutic protocols with propolis tincture (P) to reduce certain symptoms of respiratory diseases. The phytochemical composition was also determined, in order to establish a correlation between the chemical composition and the already mentioned beneficial effects obtained upon administration. The results revealed an important amount of polyphenolic compounds for the propolis tincture (P) and consequently a very good antioxidant and antibacterial activity. Regarding the glycerinic extracts, RC and BP were the richest in total polyphenols and flavonoids, while the VL extract was the poorest in all analysed compounds. RC extract was the only one containing all three analysed triterpenes, with lupeol identified in the highest concentration. The P exhibited the best antibacterial activity, against all tested bacterial strains.

Rezumat

Scopul principal al studiului a constat în testarea proprietăților antioxidante și antimicrobiene ale unor preparate gemoterapice obținute din mai multe specii de plante: *Betula pubescens* (BP), *Ribes nigrum* (RN), *Rosa canina* (RC), *Viburnum lantana* (VL) și *Carpinus betulus* (CB), frecvent asociate cu tinctura de propolis (P) în protocoalele terapeutice, în scopul reducerii anumitor simptome specifice bolilor respiratorii. Au fost efectuate studii fitochimice pentru a stabili existența unei corelații între compoziția chimică și cele două efecte terapeutice menționate anterior. Rezultatele cercetărilor au relevat conținutul bogat în compuși polifenolici al tincturii de propolis și consecutiv o foarte bună activitate antioxidantă și antibacteriană. Dintre preparatele gemoterapice, extractele de RC și BP s-au remarcat printr-o concentrație crescută de compuși polifenolici și flavonoide, în timp ce în extractul de VL, toți compușii analizați au fost prezenți în cantități scăzute sau nu au putut fi evidențiați. Extractul de RC a fost singurul în care a fost identificată prezența celor trei triterpene analizate, lupeolul fiind majoritar. În privința efectului antibacterian, P a exercitat un efect foarte bun asupra unor microorganisme testate: *Staphylococcucus aureus*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Escherichia coli*.

Keywords: gemmotherapic remedies, respiratory diseases, phytochemistry, LC/MS

Introduction

Gemmotherapy is a modern method of draining the organism. Gemmotherapy medicine uses herbal remedies principally obtained from embryonic tissue (buds and emerging shoots), reproductive parts (seeds and catkins), and newly grown tissue (rootlets and cortex of rootlets) of various trees and shrubs. The raw material is freshly harvested, during the plants growing stages, because the concentration in some active principles of immature plants is much higher than in mature plants. In many cases, some pharmacological active substances disappear after the plant reaches a certain stage of its development [17]. As soon as they are harvested, buds and embryonic plant tissue are soaked in a mixture of glycerine and alcohol. The proportion corresponds to $1/20^{th}$ of the dry weight of the fresh plant. After three weeks, the macerate is filtered and diluted to $1/10^{th}$ with a mixture of water, alcohol and glycerine [11, 14, 17].
The literature mentions a complex composition for embryonic tissue: growth factors or phytohormones (such as auxins and gibberellins), vitamins, enzymes, essential trace minerals elements, etc. All these compounds can be found in gemmotherapic remedies and, therefore, can be consumed by human, so a

variety of processes such as detoxification can be stimulated [17].

In order to set a scientific ground for the empirical observations of the practitioners, studies are required, in which a correlation must be established between the chemical composition and the effects upon administration. Scientific research in gemmotherapy involves chemical analysis of plant material, study of pharmacological properties and clinical assessment. The first work that demonstrated the beneficial effect of glycerine macerates of embryonic plant tissue dates from the 1950's [17].

However, at the moment, there are few data on chemical composition of buds and other embryonic tissues, so the major objective of our study was to establish if there is a positive correlation between the phytochemical profile and beneficial effects of some glycerinate macerates obtained from several plant species: *Betula pubescens* Ehrh. = BP (birch), *Ribes nigrum* L. = RN (blackcurrant), *Rosa canina* L. = RC (dog rose), *Viburnum lantana* L. = VL (wayfaring tree) and *Carpinus betulus* L. = CB (common hornbeam), often combined with *Propolis tinctura* = P in therapeutic protocols, to reduce the intensity of certain symptoms specific to some respiratory diseases, such as: viral infections, acute and chronic bronchitis, allergic rhinitis and asthma. All the plant species are cited in the literature for their anti-inflammatory, anti-allergic and antioxidant properties [2, 4, 6].

Materials and Methods

Sample preparation

The gemmotherapic remedies that we analysed are produced by Plantextrakt Laboratory, Romania. According to the information provided in the leaflet of each extract, the plant material is represented by buds of BP, RN, VL and CB and offshoots of RC. The buds and young shoots have grown in the wild or in organic cultures far from possible sources of pollution. The fresh buds or shoots are macerated in a mixture of glycerine and alcohol, with a concentration of $1/20^{th}$ of dry bud weight and the potentiation is made in a DH1 potency (mother-macerate is diluted at $1/10^{th}$ in a mixture of glycerine, alcohol and water). Glycerine is selected as an excipient as it allows a better extraction of the embryonic ingredients [17].

For the P preparation, the appropriate ratio between the mass of raw propolis and final volume of extract was established at 150 g propolis/L tincture.

Both, non-hydrolysed (n) and hydrolysed (h) extracts were analysed in order to determine the polyphenol content. The hydrolysis was performed using 2N hydrochloric acid at a temperature of 80°C for 60 minutes.

Determination of total polyphenols and flavonoid contents

The total phenolic content (TPC) of the extracts was measured using the Folin-Ciocalteu method, with some modifications. The absorbance was measured at λ = 760 nm, using a V530 JASCO UV-VIS spectrophotometer. A standard curve was prepared by using different concentrations of gallic acid. TPC was expressed as mg gallic acid/g dry material plant (mg GAE/g plant material).

The total flavonoid content (TFC) was determined and expressed as rutin equivalents (mg RE/g plant material), using the method described in the Romanian Pharmacopoeia (X^{th} Ed.) [18]. The absorbance was measured at λ = 430 nm.

LC/MS analysis of polyphenolic compounds

The qualitative and quantitative determination of polyphenols was achieved using an HPLC-MS method. The experiment was carried out using an Agilent 1100 Series HPLC system (Agilent USA) consisting of a G1322A degasser, G1311A binary gradient pump, a G1313A autosampler and a UV detector. The chromatographic separation was performed using the chromatographic previously described technical conditions [1, 7]. For the quantitative determination, the external standard method was used. The chromatographic data were processed using Chemstation and Data Analysis software from Agilent, USA. Also, the calibration curves in the 0.5 - 5 µg/mL range showed good linearity ($R^2 < 0.999$) for a five point plot [1, 3, 7].

Due to an incomplete separation of chlorogenic and caffeic acids, the quantitative and qualitative analysis of both compounds was carried out using a mobile phase consisting of 0.1% acetic acid and acetonitrile (V/V) in the previously described conditions. Although, to avoid or limit the interference from background, the multiple reactions monitoring analysis mode was used (MS/MS) [9].

Chromatographic condition for the analysis of some methoxylated flavonoids aglycones

The same liquid chromatography/tandem mass spectrometry (LC/MS/MS) equipment was used for the qualitative and quantitative determination of aglycons (jaceosidin, hispidulin, eupatilin, eupatorin, casticin and acacetin), in the previously described chromatographic conditions. The mobile phase consisted of aqueous acetic acid (0.1%, V/V) and methanol. The gradient program was as follows: the gradient started with 45% methanol, at 8 min 50% methanol.

Chromatographic conditions for the analysis of triterpenes

The triterpenic compounds were separated using a Zorbax SB-C18 (Agilent), reversed phase analytical column (100 mm x 3.0 mm i.d., 3.5 µm particle) maintained at 45°C. The same LC/MS system was used. The separation was achieved under isocratic conditions, using a mobile phase consisting of 0.4%

formic acid and methanol (V/V) for the separation of betulin and betulinic acid and 100% methanol for the separation of lupeol. The mobile phase was delivered with a flow rate of 1 mL/min with an injection volume of 2 µL for betulin and betulinic acid and 0.5 µL for lupeol. For the detection of triterpenes, single ion monitoring was used. The MS system operated using an ion trap mass spectrometer with electrospray positive ionization.

DPPH assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was used to evaluate radical scavenging activity, by bleaching of purple methanolic solution of the stable radical. The antioxidant effect is depicted by the disappearance of the DPPH absorption by the action of antioxidants. An aliquot of 20 µL of diluted extracts were added to 980 µL DPPH solution (100 µM). The absorbance was measured at λ = 517 nm. Both hydrophilic and lipophilic synthetic antioxidants, quercetin and butylated hydroxyl-toluene (BHT) were used as standards. The percentage inhibition of the DPPH radical after adding individual samples was calculated using the following equation:

$$I = 100 (A_c-A_s)/A_c,$$

where I = DPPH inhibition (%), A_c = absorbance of control sample, A_s = absorbance of tested sample. The antioxidant activity was also expressed as inhibitory concentration IC_{50}, defined as the concentration of sample required to cause a 50% decrease in initial DPPH radical absorbance. IC_{50} values in DPPH assay were calculated graphically. All experiments were performed in triplicate.

Antibacterial activity

Microorganisms and culture conditions

For the bioassay, there were used: Gram positive bacteria - *Staphylococcus aureus* (ATCC 49444) and *Listeria monocytogenes* (ATCC 19114) and Gram negative bacteria - *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922) from Food Biotechnology Laboratory, Life Sciences Institute, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania. The bacteria were cultured on Muller-Hinton agar and cultures were stored at 4°C and subcultured once a month. For the antimicrobial activity evaluation, the obtained extract was evaporated to dryness under reduced pressure at 30°C and re-suspended in 1 mL of bidistiled water.

Microdilution method

In order to evaluate the antimicrobial activity, a modified microdilution technique was used. Technical data concerning this method was already published [9]. Two parameters were determined: the minimum inhibitory concentrations (MICs) and the minimum bactericidal concentrations (MBCs). The MIC of the samples was detected following the addition of resazurin solution. A change from blue to pink indicates the reduction of resazurin and therefore bacterial growth. The MIC was defined as the lowest drug concentration that prevented this colour change. The minimum bactericidal concentrations (MBCs) were determined by serial sub-cultivation of inoculum. The lowest concentration with no visible growth was defined as MBC, indicating 99.5% killing of the original inoculum. Streptomycin (Sigma P 7794, Santa Clara, CA, USA) (0.05 - 3 mg/mL) was used as positive control for bacterial growth. A 10% solution of ethanol in water was used as negative control [9].

Results and Discussion

Polyphenols analysis

The total phenolic content and total flavonoid content varied considering the natural product, with higher concentrations in P (318.49 GAE/mL and 247.14 mg RE/mL, respectively); RC and BP were the richest in total polyphenols (21.88 GAE/mL and 19.54 GAE/mL, respectively) and flavonoids (17.65 RE/mL and 16.31 RE/mL, respectively), followed by RN, CB and VL extracts with comparable amounts. The concentrations of total polyphenols (12.07 - 318.49 mg GAE/mL) and flavonoids (6.18 - 247.14 mg RE/mL) are presented in Table I.

Table I

TPC and TFC in the analysed extracts (± SD)

Sample	TPC (mg GAE/mL)	TFC (mg RE/mL)
P	318.49 ± 11.28	247.14 ± 9.97
RN	15.26 ± 1.07	8.93 ± 0.81
BP	19.54 ± 1.25	16.31 ± 1.27
CB	13.86 ± 0.99	6.55 ± 0.77
RC	21.88 ± 1.57	17.65 ± 1.02
VL	12.07 ± 1.05	6.18 ± 0.52

Previous work on *Rosa canina* has shown that the fruits are well known to contain a large amount of vitamin C and polyphenolic compounds, with good antioxidant effects [8]. These studies analysed the samples obtained with different solvents and extraction methods.

The LC/MS polyphenolic profile was determined using standards: phenolic acids (caffeic, chlorogenic, caftaric, gentisic, ferulic, sinapic, *p*-coumaric acids), quercetin glycosides (quercitrin, hyperoside, rutin, isoquercitrin) and flavonol and flavone aglycones (quercetin, myricetin, fisetin, patuletin, luteolin, kaempferol and apigenin).

Table II summarized the results obtained for the quantitative determination. The concentrations of identified polyphenols are organized in order of their retention times. Also, MS data and retention times were compared to those of the reference standards (n-non-hydrolysed sample, h-hydrolysed sample).

Table II

The polyphenolic compounds (μg/mL) determined in the tested samples

Polyphenolic compound	m/z	R_T ± SD (min)	P n/h	RN n/h	BP n/h	CB n/h	RC n/h	VL n/h
p-Coumaric acid	163	9.48 ± 0.08	196.48	13.74	56.13	NF	1.46	NF
			222.48	11.03	46.74	2.68	9.34	NF
Ferulic acid	193	12.8 ± 0.10	154.0	6.82	3.33	NF	NF	NF
			198.19	6.27	6.97	NF	NF	NF
Sinapic acid	223	14.3 ± 0.10	NF	0.71	NF	NF	NF	NF
			NF	0.17	2.18	NF	NF	NF
Hyperoside	463	19.32 ± 0.12	NF	19.99	70.46	4.12	28.39	NF
			NF	NF	NF	NF	NF	NF
Isoquercitrin	463	19.60 ± 0.10	NF	71.54	16.11	NF	22.54	NF
			NF	NF	NF	NF	NF	NF
Rutin	609	20.20 ± 0.15	NF	27.57	1.74	NF	14.36	NF
			NF	NF	NF	NF	NF	NF
Myricetin	317	21.13 ± 0.12	4.58	2.27	NF	NF	NF	NF
			3.32	11.14	0.26	NF	0.97	NF
Quercitrin	447	23.64 ± 0.13	1.47	11.58	10.82	19.42	147.87	NF
			NF	NF	NF	NF	NF	NF
Quercetin	301	26.80 ± 0.15	14.11	5.29	4.31	NF	2.98	NF
			4.09	44.16	29.52	3.1	76.37	NF
Luteolin	285	29.10 ± 0.19	2.59	0.46	NF	NF	NF	NF
			1.21	NF	NF	0.38	NF	NF
Kaempferol	285	32.48 ± 0.17	14.49	1.14	57.08	NF	0.35	NF
			2.02	7.3	14.23	0.56	16.66	NF
Apigenin	279	33.10 ± 0.15	9.78	NF	22.33	NF	NF	NF
			5.471	NF	8.80	NF	NF	NF

Note: NF - not found, below the limit of detection. Each value is the mean ± SD of three independent measurements

Table III

Quantitative determination of caffeic and chlorogenic acids

Sample	Caffeic acid (μg/mL)	Chlorogenic acid (μg/mL)	Specific ions for identification (m/z)
P	282.70	3.12	Caffeic acid: m/z 179 > m/z 135 Chlorogenic acid: m/z 353 > m/z 191
RN	18.43	16.09	
BP	1.38	13.90	
CB	NF	5.44	
RC	1.48	41.00	
VL	0.17	1.67	

Considering the results summarised in Table II and Table III, the propolis tincture was the richest in phenolic acids. p-Coumaric, ferulic, caffeic and chlorogenic acids were identified and quantified, with concentrations of 196.48 μg/mL, 154 μg/mL, 282.7 μg/mL and 3.12 μg/mL, respectively. The concentration level of *p*-coumaric and ferulic acids increased through hydrolysis, probably due to the fact that in non-hydrolysed samples both phenolic acids were esterified.

Except CB, caffeic acid was quantified in all analysed samples. The results revealed the presence of chlorogenic acid in all extracts. The highest concentration was quantified in RC extract (41 μg/mL), while the lowest concentration was determined in VL extract (1.67 μg/mL).

Sinapic acid was identified in both non-hydrolysed and hydrolysed extract (n/h) of RN and in the hydrolysed extract of BP, but the amount was low. None of the other samples was found to contain sinapic acid.

Aglycones were identified and quantified: myricetin, quercetin, luteolin, kaempferol and apigenin. The results revealed that kaempferol was the most abundant aglycone. Except VL, in all the other samples the concentration of kaempferol varied over a wide range (0.35 - 57.08 μg/mL). We also noticed the presence of myricetin in P and RN (Table IV).

Quercitrin and hyperoside were the most predominant, with concentrations comprised between 1.47 - 147.87 μg/mL and 4.12 - 70.46 μg/mL. Quercitrin appears in the highest concentration in RC, while hyperoside is predominant in BP (Figure 1).

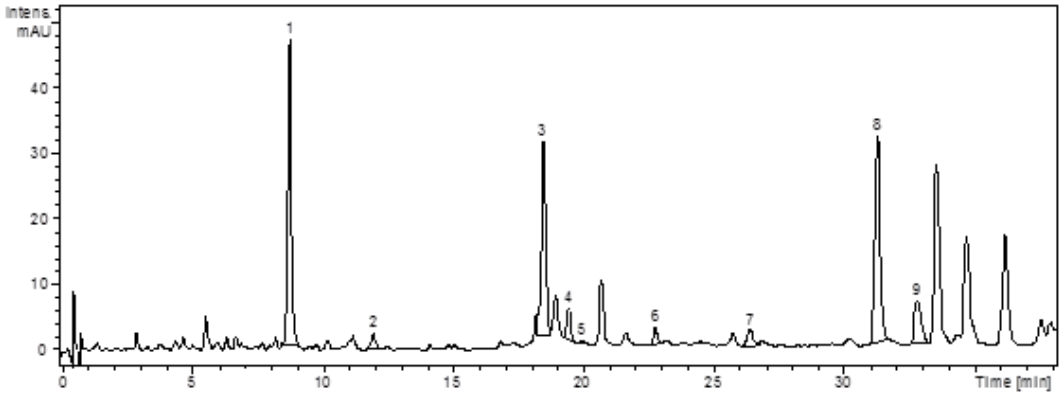

Figure 1.

The UV chromatogram of *Betula pubescens* gemmotherapic extract: 1, p-Coumaric acid; 2, Ferulic acid; 3, Hyperoside; 4, Isoquercitrin; 5, Rutin; 6, Quercitrin; 7, Quercetin; 8, Kaempferol; 9, Apigenin

Table IV

Quantitative determination of methoxylated flavonoid aglycones

Sample	Eupatorin (µg/mL)	Eupatilin (µg/mL)	Acacetin (µg/mL)	Jaceosidin (µg/mL)	Casticin (µg/mL)	Hispidulin (µg/mL)
	m/z 328	m/z 328	m/z 268	m/z 314	m/z 358	m/z 284
P	NF	NF	331.50	654.49	NF	266.12
RN	NF	NF	311.66	NF	NF	84.01
BP	541.36	NF	39795.72	NF	146.28	4831.73
CB	NF	NF	NF	205.26	NF	NF
RC	35.46	NF	NF	NF	111.27	60.22
VL	NF	NF	NF	NF	NF	NF

Note: NF-not found, below the limit of detection. Each value is the mean ± SD of three independent measurements

Except VL, the methoxylated aglycones were identified in all analysed samples, in variable quantities. None of the samples was found to contain all the analysed compounds. Eupatilin was not identified in any of the tested extracts. The most abundant aglycone was hispidulin, the tests revealing its presence in four samples. BP is the richest in methoxylated flavonoid aglycones. The highest amount was obtained for acacetin and hispidulin, with concentrations of 39795.72 µg/mL and 4831.73 µg/mL, respectively. In P there were identified and quantified: acacetin, jaceosidin and hispidulin, with concentrations of 331.5 µg/mL, 654.49 µg/mL and 266.12 µg/mL, respectively.

Table V

Quantitative determination of triterpens

Sample	Betulin (µg/mL)	Betulinic acid (µg/mL)	Lupeol (µg/mL)
	m/z 425.3	m/z 439.6	m/z 409.6
P	5.51	18.04	NF
RN	NF	NF	NF
BP	NF	NF	NF
CB	0.14	NF	0.025
RC	1.48	7.35	6.67
VL	NF	NF	NF

Note: NF-not found, below the limit of detection. Each value is the mean ± SD of three independent measurements

Betulinic acid and its reduced congener, betulin, exhibit a variety of medicinal and biological properties, such as: anti-malarial, antihelmintic, antinociceptive, antibacterial, anticancer and anti-inflammatory activities [10]. Lupeol has a beneficial activity against renal and hepatic toxicity, heart diseases, inflammation and diabetes [15]. Betulin, betulinic acid and lupeol, the three analysed triterpenes, were not identified in BP, RN and VL. Regarding the birch buds extract, the results are not surprising, considering the information provided by the literature. Thus, birch bark (*Betulae cortex*) is well known for its content in pentacyclic triterpenes, with the main triterpenes being betulin, betulinic acid, oleanolic acid and their derivatives lupeol and erythrodiol [3]. An inappreciable amount of betulin and betulinic acid was found in P, with concentrations of 5.51 µg/mL and 18.04 µg/mL, respectively, although lupeol was not found in the same extract. RC extract was the only one containing all three analysed triterpenes, with lupeol identified in the highest concentration (6.67 µg/mL). The same research group tested the anti-inflammatory activity of the three triterpene acids (data not shown) and they concluded that their concentration in the analysed samples was too low to significantly contribute to the observed anti-inflammatory effect (Table V).

Antioxidant activity assay

In order to evaluate the ability of gemmotherapic remedies and synthetic antioxidants quercetin and butylated hydroxytoluene (BHT) to donate the

hydrogen atom, the stable free radical DPPH was used. All samples were able to reduce DPPH radical with different degrees of intensity, depicting a scavenging activity. A lower IC_{50} value represents a higher bleaching effect, thus a better antioxidant activity.

The results obtained for the evaluation of the antioxidant activity using the DPPH bleaching assay are presented in Table VI.

Table VI

Antioxidant activity of gemmotherapic extracts and propolis tincture

Sample	IC_{50} (µg/mL)	Sample	IC_{50} (µg/mL)
P	2.79 ± 0.08	RC	5.81 ± 0.12
RN	19.52 ± 1.28	VL	30.08 ± 2.14
BP	16.39 ± 1.46	Quercetin	5.59 ± 0.13
CB	28.71 ± 1.84	BHT	15.88 ± 1.06

Note: Each value is the mean ± SD of three independent measurements

The strongest antioxidant effect was registered for P (IC_{50} = 2.79 µg/mL), with better effects. All analysed gemmotherapic extracts showed lower DPPH scavenging activity than both quercetin and BHT, except RC. The highest radical scavenging activity was determined for RC (IC_{50} = 5.81 ± 0.12 µg/mL), with positive correlation between the scavenging activity on DPPH and total phenolic content and total flavonoid content. The results could be related to the presence of higher amounts of phenolic compounds and vitamin C and indicates that the phytochemicals contribute to antioxidant properties of natural products [8, 13].The different antioxidant activities between these extracts may be due to the variability of composition and content in various active compounds, and also to the synergy between natural substances.

Considering the results, the following order in antioxidant effects was established: VL < CB < RN < BP < BHT < RC < quercetin < P. According to this method, gemmotherapic extracts developed strong antioxidant effects.

These findings are in agreement with Daels-Rakotoarison *et al.* [5], who reported IC_{50} values of 5.73 mg/L, 1.33 mg/L and 2.34 mg/L, respectively, for superoxide anion, hypochlorous acid and hydrogen peroxide methods using cell-free models for the evaluation of the antioxidant activity. The pro-anthocyanidins and flavonoids contained in *Rosa canina* fruits possess radical scavenging properties.

The rose hip extract activities were higher than other reference antioxidants (2-mercaptoethane sulphonate (mesna) and N-acetylcysteine) against HOCI and H_2O_2.

Antimicrobial activity assays

In vitro antibacterial potential is shown with the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of tested gemmotherapic extracts, P and streptomycin against bacterial strains tested by the microdilution method (Table VII).

Table VII

Results of antibacterial effect *in vitro*

Bacterial strain	Standard antibiotic	MIC/MBC(mg/mL)					
	Streptomycin	P	RN	BP	CB	RC	VL
Staphylococcus aureus	0.03	0.014	0.93	0.23	1.87	0.46	7.5
	0.06	0.029	1.87	0.46	3.74	0.93	15
Listeria monocytogenes	0.015	0.014	1.87	0.93	7.5	0.11	7.5
	0.03	0.029	3.75	1.87	15	0.23	15
Pseudomonas aeruginosa	0.06	0.23	0.93	0.93	0.93	0.93	1.87
	0.03	0.46	1.87	1.87	3.74	1.87	7.5
Escherichia coli	0.12	0.46	1.87	1.87	3.75	3.75	3.75
	0.12	0.93	3.75	3.75	7.5	7.5	7.5

Note: Each value is the mean ± SD of three independent measurements

There are significant differences between anti-bacterial activities of the analysed gemmotherapic extracts. P exhibited the best antibacterial activity, against all tested bacterial strains: against *Staphylococcus aureus*, (MIC = 0.014 mg/mL and 0.03 mg/mL), while the same extract exhibited a comparable activity to standard antibiotic against *Listeria monocytogenes*, (MIC = 0.014 mg/mL and 0.015 mg/mL). Also, P presented a good activity against the bacterial strains *Pseudomonas aeruginosa* and *Escherichia coli*, (MIC = 0.23 mg/mL and 0.46 mg/mL).

The tests showed that BP extract presents a good activity against *Staphylococcus aureus* strain, better than RC (MIC = 0.23 mg/mL and 0.46 mg/mL). However, *Rosa canina* extract exhibited a very good antibacterial activity against *Listeria monocytogenes.* According to Salvat *et al.* [16], if MIC values of plant extracts are less than/or around 0.5 mg/mL, the antibacterial activity is good, so we concluded that *S. aureus* is the most sensitive bacterial strain to the action of P, BP, RN and RC also exhibited a good growth inhibitory effect against *Listeria monocytogenes*. Raiciu A. *et al.* (2010) determined the antibacterial action of gemmotherapeutical extracts - Hofigal on the same bacterial strains and fungus and the results are comparable [12].

Conclusions

The small number of scientific studies dealing with the precise composition of gemmotherapic remedies makes the interpretation of results generally difficult. However, our research proves that there is a positive correlation between the phytochemical composition of analysed extracts and the therapeutic properties for which they are used (antibacterial and antioxidant effects). Associating the analysed gemmotherapic extracts with propolis tincture is beneficial and indicated, since the complex chemical composition of the latter contributes to the enhancement of its effects, through its increased content of polyphenols, flavonoids and triterpenes.

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