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Biological activity and composition of teas and tinctures prepared from *Rosa rugosa* Thunb.

Research Article

Marta Olech[1], Renata Nowak[1,*], Renata Los[†,2], Jolanta Rzymowska[3], Anna Malm[2], Katarzyna Chrusciel[1]

[1]*Department of Pharmaceutical Botany, Medical University, 20-093 Lublin, Poland*

[2]*Department of Pharmaceutical Microbiology, Medical University, 20-093 Lublin, Poland*

[3]*Chair and Department of Biology and Genetics, Medical University, 20-093 Lublin, Poland*

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Abstract: The study was designed to determine the total phenolic, flavonoid, *o*-dihydroxyphenol, tannin, and carotenoid content as well as the antiradical, antitumor and antimicrobial properties of two types of galenic preparations from *Rosa rugosa* Thunb. Such extracts obtained from various plant parts have not been studied to date. Our findings have revealed high antiradical activity of the examined galenic preparations, with root, leaf and flower extracts (IC_{50} ranging from 0.27 to 0.19 mg of dry extract per mg $DPPH^{\bullet}$) showing the greatest potential. MIC and MBC values against 8 reference bacterial strains (*i.e. Staphylococcus epidermidis, Staphylococcus aureus, Bacillus subtilis, Micrococcus luteus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis*) were determined. Generally, tinctures were found to be more active than teas with MIC ranging from 0.08 to 2.5 mg mL^{-1} and 0.31 to 1.25 mg mL^{-1}, respectively. Anticancer activities against ovarian (TOV-112D), cervical (HeLa), breast (T47D) and lung cancer (A549) cell lines were evaluated using the BrdU test. The data obtained demonstrate considerable impact of polyphenols on the anticancer activity of extracts (ethanolic, in particular).

Keywords: *Rosa rugosa* • *Radical scavenging activity* • *Antibacterial activity* • *Anticancer activity* • *Phenolic content* • *Carotenoid content*

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1. Introduction

Rugosa rose (*Rosa rugosa* Thunb.) is an Asian indigenous plant with a long record of traditional medicinal use. Since its introduction to Europe, the hips and flowers of rugosa rose and its hybrids have been widely available and used for production of teas, wines, juices and jams [1,2]. Rose hips are rich in many vitamins, which have prompted their use in medicine, particularly for conditions accompanied by immune deficiency, e.g. common cold [3]. Despite the great commercial potential of the *R. rugosa*, only a few reports have been published about the phytochemical content and biological activity of this plant; in particular, the knowledge of its roots and leaves remains limited, which is surprising considering that rose roots have been utilized by Asian folk medicine to treat conditions such as diabetes mellitus, pain and chronic inflammatory diseases [4].

Previous research has revealed the presence of many biologically active compounds in all parts of *R. rugosa* [1,4-7]. Many of them act as potential natural antioxidants [8-10]. Although some *R. rugosa* extracts were reported to possess antioxidant properties [5,11], there are no detailed studies on their antiradical potential. Similarly, there are only a few data on antibacterial and antitumor properties of the plant. Petals have been shown to have selective antibacterial activity against intestinal and pathogenic bacteria [12]. In terms of antitumor activity, Lee *et al*. [13] demonstrated that methanolic extracts from rose stem acted as anti-prostate cancer agents. The antiproliferative activity of juice from *R. rugosa* fruit on several cancer cell lines was indicated as well [14]. However, there are no precise data about phenolic content in different parts of this plant. Research on the composition and activity of other rose species may suggest that in this respect rugosa rose is also of great importance and potential [3].

* *E-mail: renata.nowak@umlub.pl;* † *deceased*

In view of several ethnopharmacological and medicinal uses and the nutritional value of the roses described in literature, it was decided to assess two types of galenic preparations (infusions and tinctures) from *R. rugosa* for *in vitro* antiradical, antimicrobial and antitumor activity with regard to their chemical composition using standard procedures. The preparations are easy to obtain in household settings and commonly used in herbal medicine. Furthermore, the method of plant material extraction used are recommended by several Pharmacopoeias, e.g. European Pharmacopoeia and Polish Pharmacopoeia [15,16]. The extracts obtained from each part of the plant have not been investigated to date and this study would represent the first wide-ranging assessment of biological activity and composition of *R. rugosa*.

2. Experimental Procedures

2.1 Plant material and preparation of extracts

Flowers, leaves, hips, nuts and roots of *R. rugosa* were collected in Serock near Lublin in Eastern Poland in 2009. The flowers and leaves were picked during blossoming time. Hips (pseudofruits) were collected in August and the nuts were separated from the red hypanthium. Roots were collected in October. The plant specimen was authenticated by Prof. Tadeusz Krzaczek and deposited at the Department of Pharmaceutical Botany, Medical University of Lublin, Poland. The plant material was dried at ambient temperature and powdered according to the European Pharmacopoeia 6th edition [17].

Infusions (teas) and ethanolic tinctures were prepared from all the collected parts of *R. rugosa* according to the monographs of the Polish Pharmacopoeia 6th edition [16]. For tea preparations, 100 mL of boiling water was poured over 2 g of pulverized plant material and left to cool; teas were filtered and filled up with distilled water to the volume of 100 mL.

Tinctures were prepared from 10 g of pulverized plant material extracted with 50 g of 70º ethanol and left in a closed glass container for seven days at ambient temperature (the mixtures were stirred occasionally three times a day). After this time tinctures were filtered and filled up with 70º ethanol to the measured volume.

30 mL of each fresh extract was used for analysis of chemical composition. The remaining amount was lyophilized in Free Zone 1 apparatus (Labconco, Kansas City, KS, USA), then weighed and stored in the refrigerator. Extracts re-dissolved in the appropriate solvents were used in determinations of cytotoxic, antibacterial and antiradical activities.

All extracts were prepared in duplicate and the obtained samples were marked with the following symbols: D – infusions (teas), T – tinctures, Flos – flower, Fol – leaf, P – pseudofruit (hip), N – nut, Rx – root; e.g. D Flos means tea prepared from flowers.

2.2 Chemicals

Gallic acid, caffeic acid, quercetin, pyrogallol, Trolox, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$), and hide powder were purchased from Sigma–Aldrich Fine Chemicals (St. Louis, MO, USA). Hyperoside was from LGC standards (Dziekanów Leśny, Poland) and β-carotene was from Fluka (Buchs, Switzerland). Ethanol, methanol, sodium molybdate, sodium nitrite, dimethyl sulfoxide (DMSO) and Folin–Ciocalteu phenol reagent were from POCh (Gliwice, Poland). All the chemicals used were of analytical grade.

All the spectrophotometric assays were conducted in the Thermo Scientific Evolution 300 spectrophotometer (Lafayette, CO, USA).

2.3 Total phenolic content

The total phenolic content (PC) was determined according to the Singleton and Rossi method, with some modifications [5,18]. Briefly, 1 mL of the examined extract was added to 1 mL of diluted Folin–Ciocalteu phenol reagent dissolved with water (1:5, v/v). The mixture was filled up with sodium carbonate solution (75 g L^{-1}) to the volume of 10 mL. Absorbance at 660 nm was measured after 20 min. The results were expressed as mg of gallic acid per mL of galenic preparation as well as per typical administered dose (*i.e.* per 250 mL tea or per 25 mL tincture dose).

2.4 Total flavonoid content

The flavonoid content was determined colorimetrically according to the method described by Lamaison and Carret [19]. 1 mL of sample was mixed in a test tube with 1 mL of 2% (w/w) $AlCl_3$ methanolic solution and filled with methanol to the volume of 10 mL. Absorbance was estimated at 394 nm after 10 minutes (for leaf extracts at 430 nm). The total flavonoid content was expressed as mg of hyperoside per 1 mL and per serving of galenic preparation.

2.5 Determination of *o*-dihydroxyphenols

In order to evaluate the content of *o*-dihydroxyphenols (DPC, caffeic acid type), one milliliter of the sample was mixed with 5 mL of distilled water, 1 mL 0.5 M HCl, 1 mL of Arnov reagent (10 g sodium molybdate and 10 g sodium nitrite in 100 mL of distilled water), 1 mL 1 M NaOH and filled up to 10 mL with distilled water. Absorbance was measured at 490 nm. The *o*-dihydroxyphenols content

was calculated using a reference curve plotted for caffeic acid and expressed as mg of caffeic acid per mL of extract [20].

2.6 Tannin content

Determination of tannin content in teas was performed according to the method described in the Polish Pharmacopoeia 6th edition with some modifications [16]. Tannins were estimated indirectly after adsorption on, and precipitation with, insoluble hide powder. First, 2 mL of tea diluted with water (1:5, v/v) was mixed with 1 mL of Folin–Ciocalteu reagent, 10 mL of water and 12 mL of sodium carbonate solution (290 g L^{-1}) and the absorbance (A_1) was measured at 760 nm after 30 minutes against a water blank to estimate the total phenolic content. The hide powder was then added to the original extract (10 mg hide powder per mL original extract) and the mixture was thoroughly mixed and centrifuged (2900×*g*, 10 min) to precipitate tannins. Total phenols were measured in the supernatant after dilution as described above (absorbance A_2). The amount of extractable tannin (ET) was calculated as follows:

$ET=A_1-A_2$ and expressed as mg of pyrogallol per mL of extract with a reference curve plotted for this standard.

2.7 Total carotenoid content

The carotenoids in galenic preparations were determined spectrophotometrically by means of the modified method described in Current Protocols in Food Analytical Chemistry [21]. In short, 2 mL of each galenic preparation was mixed with 2 mL of hexane and shaken for 5 minutes in the dark. The mixture was centrifuged at 2900x*g* for 5 min at 4ºC. The absorption of hexane extracts was measured at 460 nm against hexane. Results were expressed as mg of β-carotene (reference standard) per mL and per typical dose of galenic preparation.

2.8 *In vitro* cytotoxic assay

Teas and tinctures prepared from *R. rugosa* were evaluated for their anticancer activities against ovarian (TOV-112D ATCC CRL-11731 human ovary primary malignant adenocarcinoma, endometrioid carcinoma cell line), and lung cancer (A549 ECACC 86012804 human lung epithelial cell line derived from a 58-year-old Caucasian male) cell lines. Additionally, preliminary screening of anticancer properties of teas against cervical (HeLa ECACC 93021013 human cervical epithelial cell line derived from cervical carcinoma of a 31-year-old patient) and breast cancer (T47D ECACC 85102201 human breast carcinoma cells) was carried out. Normal human skin fibroblast cells *in vitro* (HSF primary cell line isolated from the skin of a 25-year-old female 5th passage) were included in the cytotoxicity test as a control group.

In the present protocol, each cell line was inoculated at 10^4 cells per mL density on a microtiter plate (Nunc, Roskilde, Denmark) in the RPMI 1640 medium (Sigma, St. Louis, MO, USA) with 10% FBS (Sigma) and the extracts added at concentration of 100 µg mL^{-1}. The cultures were incubated for 48 and 72 h under standard conditions (37°C, 5% CO_2, 90% humidity). End-point determinations were performed with 5-bromo-2-deoxy-uridine (BrdU) labeling and the detection kit III (Roche Diagnosis, Mannheim, Germany) using an Elisa reader (Genesys 20, Thermo Spectronic, Madison, WI, USA) [22].

The growth percentage was evaluated spectrophotometrically *versus* the untreated controls using the cell viability assay. The results of each spectrophotometric measurement were expressed as the percentage of cells with growth inhibition compared to untreated samples. All experiments were carried out in triplicate.

2.9 Antibacterial assay *in vitro*

The antibacterial potential of teas and tinctures of *R. rugosa* has been evaluated using the micro-broth dilution method, which enables determination of minimal inhibitory concentration (MIC) according to the early described procedure [23]. The eight reference strains, including Gram-positive bacteria (*Staphylococcus epidermidis* ATCC 12228, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Micrococcus luteus* ATCC 10240) and Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 9027, *Proteus mirabilis* ATCC 12453) were used. The plant material was dissolved in dimethyl sulfide (DMSO) and the series of their two-fold dilutions, ranging from 0.02 to 2.5 mg mL^{-1}, was prepared in Mueller-Hinton broth (Biocorp, Poland) in 96-well microtiter plates. The wells were inoculated with the bacterial suspension (the final inoculum size of 10^6 colony forming units – CFU mL^{-1}). Following the 24-hour incubation in air at 35ºC, the wells were inspected for bacterial growth and MIC was defined as the lowest concentration of the extract which did not show visual growth. DMSO was used as a negative control. Controls containing only plant material in broth and broth with inocula were also included. Gentamicin was used as a reference compound. Minimal bactericidal concentrations (MBCs) were determined by collecting 20 µl from each well with growth inhibition and placing onto duplicate Mueller-Hinton agar plates and incubating in air at 35ºC for 24 h. MBC was defined as the lowest extract concentration at which there was no bacterial growth. The assay was performed in triplicate.

2.10 Scavenging of DPPH• radicals

The scavenging effect of galenic preparations was monitored as previously described with some modifications [5]; 1.96 mL of 0.2 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH•) coloured solution in methanol were mixed with 0.04 mL of extract. A decrease in DPPH absorbance induced by the sample was measured at 517 nm against methanol as a blank.

Preliminary analysis of inhibition of free radicals in time was performed. For this purpose samples of identical concentrations were prepared (2.5 mg dry weight mL^{-1}). A decrease in absorbance in the presence of *R. rugosa* extracts was noted every 60 s for 30 minutes.

To determine IC_{50} of active extracts, the technique using 96-well microplates was employed. Aliquots of 190 µl of a freshly-prepared 2,2-diphenyl-1-picrylhydrazyl (DPPH•) coloured solution in methanol (0.07 mg mL^{-1}) were mixed with 10 µl of the extract diluted to various concentrations in 96-well microplates. The solutions were shaken and incubated at 28°C for 60 min in the dark. A decrease in DPPH• absorbance induced by the sample was measured at 517 nm using an Elisa reader (Infinite Pro 200F, Tecan Group Ltd., Männedorf, Switzerland).

Antiradical activity of galenic preparations was calculated according to the following formula:

$$\% \text{ Inhibition} = [(A_B - A_A)/A_B]x100$$

where: A_B – absorption of a blank sample (DPPH• solution and methanol instead of the test extract), A_A - absorption of a tested sample with DPPH• reagent.

A dose response curve for five prepared dilutions of each extract was plotted to determine the IC_{50} values. IC_{50} is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity. All tests were carried out in triplicate and averaged. Results were expressed as standard equivalents using Trolox (TE), quercetin (QE), and gallic acid (GAE) based on their IC_{50} values. Moreover, the antiradical efficiency ($AE=1/IC_{50}$) was calculated.

2.11 Statistical analysis

The extracts were assayed in triplicate in each test. Data were expressed as a mean ± the standard deviation of the independent measurements. For statistical analysis, Statistica 6.0 and Excell were used. Significant differences were calculated according to the Duncan's multiple range test. Differences at the level of 5% were considered statistically significant. Correlation coefficients between the components and activities of the analyzed extracts were determined.

3. Results and Discussion

3.1 Determination of total phenolic content

In order to determine total phenolic content, the Folin-Ciocalteu method was used. Total phenols in samples varied from 0.14 to 13.9 mg of gallic acid equivalents per mL of rose extract, depending on a plant part and kind of galenic preparation (Table 1).

In the majority of cases, the analyzed rose galenic preparations were found to be abundant in phenolic compounds; the highest content being observed in flower, leaf and root ethanolic extracts, ranging from 12.75-13.9 mg mL^{-1}. Considerable amounts were found in hip tincture (11.88 mg mL^{-1}) whereas the lowest content was in nut ethanolic extract (2.88 mg mL^{-1}). Teas contained lower amounts per milliliter ranging from 0.14 to 1.52 mg of phenols for nut and flower tea, respectively. However, the amounts contained in a typically administered serving of tea and tincture from a given plant part were comparable.

Polyphenols are the most commonly studied group of plant secondary metabolites, particularly in conjunction with antioxidant activity determinations. It is well known that they have a proven impact on the extract's biological activity [24]. Thus, they are desirable components of diets and the amounts found in the analyzed preparations (especially in flower and hip preparations) are of nutritional value.

3.2 Determination of flavonoid content

Flavonoids are polyphenolic compounds naturally present in plant foods. They are currently widely studied because of their nutraceutical and health benefits. Their ability to reduce the risk of cardiovascular diseases as well as free radical scavenging activity has been well established [25].

As shown in Table 1, the highest concentrations of flavonoids were found in flower and leaf tinctures (1.14 and 1.25 mg mL^{-1}, respectively). The root ethanolic extract also contained considerable amounts (0.82 mg mL^{-1}) and the content of flavonoids in hip tincture amounted to 0.39 mg mL^{-1}. The smallest quantities were found in the nut extracts (0.12 mg mL^{-1}). The content of flavonoid in one milliliter of tea was approximately ten times lower than in the corresponding tincture from the same plant part, ranging from 0.01 mg mL^{-1} found in nuts to 0.11 mg mL^{-1} in flowers. However, the doses of flavonoids taken in a typical serving of tea and tincture from a given plant part are comparable, ranging from 2.5 to 31.25 mg for nut tea and leaf tincture, respectively.

It is known that the dietary intake of flavonoids in different countries ranges from several to tens of mg and tea beverages and red wine belong to the major

sources of these compounds in human diet [26]. Our study showed that some *R. rugosa* galenic preparations (particularly flower and leaf extracts) are also capable of providing the body with large doses of flavonoids.

3.3 Determination of *o*-dihydroxyphenols content

In order to analyze the *o*-dihydroxyphenols content, the spectrophotometric method was used [20]. According to our findings, the highest amounts were present in the root, leaf and flower tinctures (4.07, 2.61 and 2.15 mg mL^{-1}, respectively). The lowest content was found in tea made from the nuts - 0.02 mg mL^{-1}.

3.4 Determination of tannin content

Tannins are considered to be "antinutritional" components of beverages as they inhibit digestive enzymes, e.g. lipases, proteases, and glucosidases, thus affecting the bioavailability of lipids, proteins and carbohydrates from food [27]. However, this astringent property is also responsible for their antidiarrheal action which may be valued in some circumstances. Moreover, significant antioxidant, antibacterial, antimutagenic effects of tannins are known [12]. The tannin content in teas was determinated using the modified pharmacopoeial method [16]. As shown in Table 1, the amounts of tannins ranged from 0.001 to 0.225 mg mL^{-1}; the content found in root tea was high compared to the other teas.

3.5 Carotenoid content

Carotenoids, as sources of provitamin A, constitute an important group of plant nutrients. They are amongst the important antioxidant and chemopreventive constituents of rose [28]. Carotenoids protect against cardiovascular disease, neurologic conditions, inflammatory diseases, cataracts and immune disorders [29].

The carotenoid content of rose galenic preparations was determined spectrophotometrically and expressed as mg of β-carotene per mL and per typical serving of galenic preparation. Their content ranged from 0 to 9.6 μg mL^{-1} showing large variations between the different plant parts (Table 1). Our findings demonstrated high carotenoid content in raw plant material. However, the levels of carotenoids found in our study were

Galenic preparation	Phenolics mg mL^{-1} and *mg per serving ±SD	Flavonoids mg mL^{-1} and *mg per serving ±SD	Tannins mg mL^{-1} and *mg per serving ±SD	o-Dihydroxyphenols mg mL^{-1} and *mg per serving ±SD	Carotenoids μg mL^{-1} and *mg per serving ±SD
D Flos	1.52 ± 0.09	0.11± 0.01	0.06 ± 0.01	0.24 ± 0.18	0.11 ± 0.01
	380.0* ± 1.51	27.5* ± 0.28	14.48* ± 1.65	60.0* ± 0.44	27.5* ± 0.55
D Fol	1.25 ± 0.05	0.10 ± 0.01	0.058 ± 0.005	0.26 ± 0.23	0.09 ± 0.01
	312.5* ± 1.22	25.0* ± 0.25	14.48* ± 1.25	65.0* ± 0.56	22.5* ±0.29
D P	1.15 ± 0.11	0.03± 0.00	0.076± 0.01	0.14 ± 0.01	1.1 ± 0.08
	287.5* ± 1.15	7.5* ± 0.75	19.05* ± 0.5	35.0* ± 0.14	275* ±1.13
D N	0.14 ± 0.03	0.01 ± 0.00	0.001± 0.00	0.02 ± 0.00	0 ± 0.00
	35.0* ± 0.14	2.5* ± 0.01	0.23* ± 0.00	5.0* ± 0.02	0* ± 0.00
D Rx	1.45 ± 0.09	0.08 ± 0.00	0.225 ± 0.03	0.32 ± 0.08	0.17 ± 0.02
	362.5* ± 1.48	20.01* ± 0.26	56.25* ± 7.5	80.0* ± 0.72	42.5* ± 0.17
T Flos	12.93 ± 0.14	1.14 ± 0.01	-	2.15 ± 0.14	5.27 ± 0.44
	323.25* ± 3.92	28.5* ± 2.5	-	53.75* ± 2.15	131.75* ± 1.27
T Fol	12.75 ± 0.10	1.25 ± 0.01	-	2.61 ± 0.05	1.95 ± 0.14
	318.75* ± 2.75	31.25* ± 2.32	-	65.25* ± 2.61	48.75* ± 0.95
T P	11.88 ± 0.1	0.39 ± 0.06	-	0.84 ± 0.02	9.6 ± 0.09
	297.0* ± 1.88	9.75* ± 1.25	-	21.0* ± 0.84	240.0* ± 2.6
T N	2.88 ± 0.03	0.12 ± 0.02	-	0.641 ± 0.07	0.37 ± 0.03
	72.0* ± 2.88	3.0* ± 0.24	-	16.0* ± 0.64	9.25* ± 0.37
T Rx	13.9 ± 0.29	0.82 ± 0.04	-	4.07 ± 0.04	1.4 ± 0.04
	347.5* ± 3.9	20.5* ± 2.06	-	101.75* ± 4.07	35.0* ± 1.4

Table 1. Total phenolic, flavonoid, tannin, o-dihydroxyphenol and carotenoid contents in different *R. rugosa* teas and tinctures. Mean values of three replicate assays with standard deviation. Abbreviations: D – infusion (tea); T – tincture; Flos – flower; Fol – leaf; P – pseudofruit (hip); N – nut; Rx – root. One serving = 250 mL for teas and 25 mL for tinctures.

slightly lower than those reported by other authors [6]. The differences might be related to environmental and genetic factors, or may be due to different methods of extraction and analysis, particularly that the extraction methods used to obtain galenic preparations are not exhaustive.

The best sources of carotenoids were hip extracts (240 and 275 µg per serving for tincture and tea, respectively) and flower tincture with 131.75 µg per serving. These galenic preparations are readily accessible and may be consumed in sufficient amounts and frequencies in order to meet dietary needs.

3.6 Antitumor activity

Antitumor activities of teas and tinctures prepared from *R. rugosa* against ovarian (TOV-112D), lung cancer (A549) cell lines as well as normal human skin fibroblasts were evaluated. Our study revealed potent cytotoxic properties of almost each rose extract analyzed (Figure 1); the ability to destroy cancer cell lines without damaging fibroblasts is of particular interest. DRx and TN extracts at 100 µg mL^{-1} had high antitumor effects on human lung (80 and 95% of dead cells, respectively), ovary cell lines (95 and 75% of dead cells, respectively) with a marginal toxic effect (5%) on normal skin fibroblast cell lines. Similar effects were noticed after incubation of human lung and ovary cell lines with DP at the same dose; however, DP exhibited more selected anticancer effect on the TOV-112D line (80% of dead cells). Moreover, the activity of hip tinctures on lung cancer lines is noteworthy (55% of dead cells).

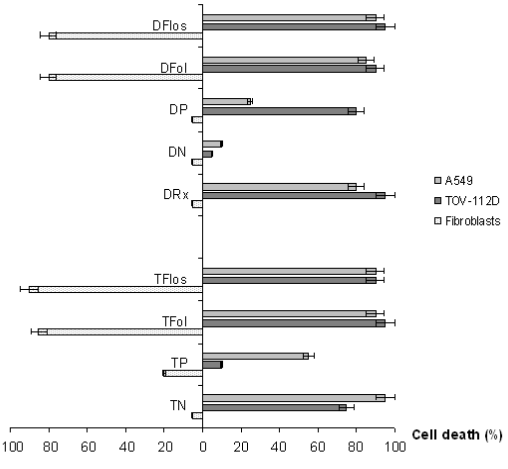

Figure 1. Results of cell proliferation assay obtained for *Rosa rugosa* Thunb. teas and tinctures added at concentration of 100 µg mL^{-1}, after 48 h incubation. Data are expressed as the percentage of dead cells. Error bars indicate the standard deviation. Antiproliferative activity of root tincture was not evaluated. Abbreviations as in Table 1.

Tinctures and decoctions prepared from flowers and leaves showed a considerable antitumor effect on A549 and TOV-112D lines (85% to 95% of dead cells), yet the damaging effect on normal fibroblast cell lines was also significant (80% to 90%).

Additionally, preliminary screening of tea anticancer activity against cervical (HeLa) and breast cancer (T47D) was conducted, showing a significant viability decrease in both lines after exposure to DRx (50% and 75% for HeLa and T47D, respectively). The significant cytotoxic influence (50% of dead cells) was observed after incubation of the cervical cell line (HeLa) with DFlos and breast cancer line (T47D) with DN. A 25% viability decrease in T47D line after incubation with DFlos and HeLa line with DN was noticed. Teas obtained from leaves and hips did not show any significant anticancer effect. Noteworthy, cytotoxic properties of *R. rugosa* against ovarian (TOV-112D), lung (A549), cervical (HeLa) and breast cancer (T47D) have been studied for the first time.

3.7 Antibacterial activity

All the plant extracts from *R. rugosa* were assessed for their antibacterial properties against the reference Gram-positive and Gram-negative bacteria. The resulting data are presented in Table 2. Our results showed that both the teas and the tinctures were active against all the bacteria used. However, the power of bacterial growth inhibition depended on the kind of rose galenic preparations, the part of the rose plant used for extraction, and the bacterial species. In most cases tinctures were found to be more active than teas with MICs ranging from 0.08 to 2.5 mg mL^{-1} and 0.31 to 1.25 mg mL^{-1}, respectively. Only *P. aeruginosa* was more sensitive to the majority of tested teas compared to tinctures (MICs 0.31 to 0.62 mg mL^{-1} and 0.63 to 1.25 mg mL^{-1}, respectively), except for root galenic preparations which showed a similar inhibitory effect (MIC=0.63 mg mL^{-1}). The rose hip tea demonstrated stronger antistaphylococcal potency (MICs 0.63 and 1.25 mg mL^{-1}) than rose hip tincture (MIC 1.25 and 2.5 mg mL^{-1}). Similar sensitivity to both galenic preparations was observed for *P. mirabilis*, irrespective of the part of plant used for extraction with MICs ranging from 0.63 to 1.25 mg mL^{-1} and for *E. coli* and *K. pneumonie* in the case of nut and pseudofruit extracts (MIC=1.25 mg mL^{-1}). *M. luteus* was the most sensitive species to both rose galenic preparations with MIC ranging from 0.08 to 0.63 mg mL^{-1}. Both tinctures and teas sampled from leaves showed the best antibacterial properties, followed by flowers and roots, whereas nut and pseudofruit samples exhibited lower antibacterial activity. Furthermore, the activity of plant materials was

examined in detail determining the minimal bactericidal concentration (MBC) and comparing with MIC values. It is worth emphasizing that low MBC/MIC ratios (≤4) indicate bactericidal activity. According to our data (Table 2), all tested teas and most tinctures were bactericidal, except for flower extracts towards Gram-positive bacteria (MBC/MIC ratio = 8 for *S. aureus*, *B. subtilis* and *M. luteus*). Moreover, root, leaf and fruit tinctures showed bacteriostatic activity for *M. luteus* (MBC/MIC ratio from 8 to 16).

To sum up, such detailed studies on antimicrobial properties of *R. rugosa* have not been conducted previously. All rose galenic preparations exerted from good to moderate effects against Gram-positive and Gram-negative bacteria. However, antimicrobial properties of tinctures were found to be superior to those of infusions. The best antibacterial properties were found in samples from leaves, followed by flowers and roots, whereas nut and pseudofruit samples exhibited lower antibacterial activity. According to our findings, rose galenic preparations have potential as mucous membrane antiseptic agents for the upper respiratory or alimentary tract; the leaf tinctures in particular showed good bactericidal effect and *in vitro* activity.

3.8 Antiradical activity analysis

Analysis of DPPH$^{\bullet}$ scavenging activity was performed using the method previously described with some modifications [5]. The results of the preliminary antiradical analysis were presented as graphs illustrating the kinetics of reactions. All of the extracts demonstrated high free radical scavenging activity (Figure 2). *R. rugosa* flower extracts were the most potent radical scavengers. Leaves and roots had almost equal effects, neutralizing all free radicals within 15 minutes. The slowest were the preparations obtained from *R. rugosa* nuts and hip tinctures, which needed approximately twofold longer periods of time to cause similar DPPH$^{\bullet}$ inhibition.

Subsequently, the extract concentration that would provide 50% scavenging of the initial amount of DPPH$^{\bullet}$ radical under conditions of the assay used (IC_{50}) was calculated from the graph of inhibition percentage against extract concentration. The results are shown in Table 3. Antiradical efficiency (AE) was calculated ($AE=1/IC_{50}$). The results were also expressed as Trolox, quercetin and gallic acid equivalents. Determinations of IC_{50}, AE and equivalents values enabled precise comparison of antiradical activity of the examined extracts (Table 3) [9].

Both types of galenic preparations obtained from roots, leaves and flowers showed the highest antiradical

ATCC bacterial strains	Roots		Leaves		Flowers		Nuts		Hips	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
	Infusions									
S. epidermidis 12228	0.63	1.25	0.31	0.63	0.31	0.63	0.63	2.5	0.63	2.5
S. aureus 25923	1.25	2.5	0.63	0.63	0.63	2.5	1.25	>2.5	1.25	2.5
B. subtilis 6633	1.25	2.5	1.25	1.25	1.25	2.5	1.25	2.5	1.25	2.5
M. luteus 10240	0.63	1.25	0.63	0.63	0.63	1.25	0.63	1.25	0.63	1.25
E. coli 25922	1.25	1.25	0.63	0.63	1.25	2.5	1.25	1.25	1.25	1.25
K. pneumoniae 13883	1.25	2.5	0.63	1.25	1.25	2.5	1.25	2.5	1.25	2.5
P. aeruginosa 9027	0.63	1.25	0.31	0.63	0.63	1.25	0.63	1.25	0.63	1.25
P. mirabilis 12453	1.25	2.5	0.63	1.25	1.25	2.5	1.25	2.5	1.25	2.5
	Tinctures									
S. epidermidis 12228	0.63	1.25	0.16	0.31	0.31	0.63	0.63	2.5	1.25	>2.5
S. aureus 25923	0.63	2.5	0.31	0.62	0.31	2.5	1.25	2.5	2.5	>2.5
B. subtilis 6633	1.25	2.5	0.63	1.25	0.31	2.5	0.63	2.5	1.25	2.5
M. luteus 10240	0.16	1.25	0.08	1.25	0.08	0.63	0.31	2.5	0.31	1.25
E. coli 25922	0.63	1.25	0.63	0.63	0.63	1.25	1.25	1.25	1.25	1.25
K. pneumoniae 13883	0.63	1.25	0.31	0.31	0.63	1.25	1.25	1.25	1.25	2.5
P. aeruginosa 9027	0.63	1.25	0.63	2.5	1.25	1.25	1.25	2.5	1.25	2.5
P. mirabilis 12453	1.25	2.5	0.63	1.25	1.25	2.5	1.25	2.5	1.25	>2.5

Table 2. Antibacterial activity of teas and tinctures obtained from different parts of *R. rugosa* Abbreviations: MIC – minimal inhibitory concentration [mg mL^{-1}]; MBC – minimal bactericidal concentration [mg mL^{-1}]; ATCC – American Type Culture Collection. MICs of gentamicin ranged from 0.03-0.12x10^{-3} mg mL^{-1} and 0.25-1.0x10^{-3} mg mL^{-1} for Gram-positive and Gram-negative bacterial strains, respectively. DMSO at the final concentration used had no influence on the growth of the tested strains.

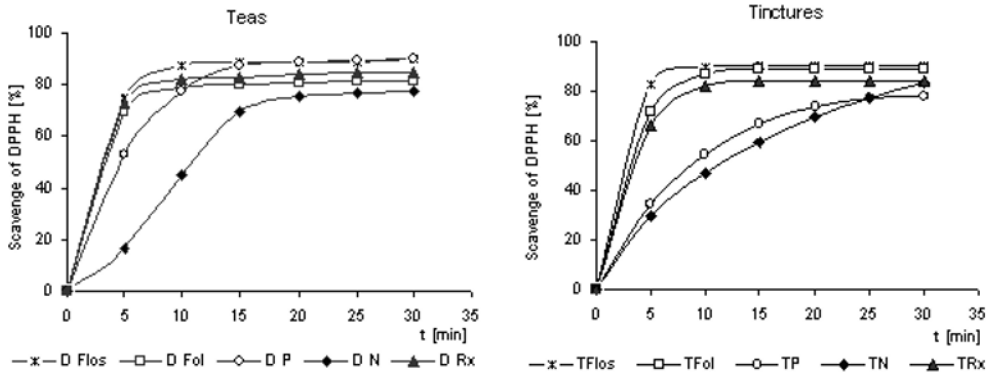

Figure 2. Dynamics of free radical scavenging activity of *Rosa rugosa* Thunb. teas and tinctures. Concentration of samples 2.5 mg dry weight mL^{-1}. Abbreviations as in Table 1.

activity. Hips and nuts had lower activities, which might be associated with limitations of the method, which is mainly suitable for analysis of polar compounds.

Although *R. rugosa* tinctures had antioxidant potential similar to the water extracts obtained from analogous plant parts, there were differences in the order of activity increase. The antiradical potential of teas increased in the following order: nuts<hips<flowers<roots<leaves, whereas the activity of tinctures increased as follows: hips<nuts<flowers<leaves<roots. It is worth noting that free radical scavenging effects of root and leaf (IC_{50} 0.19-0.22) preparations were comparable to those of Trolox (IC_{50} 0.185). The data obtained in the present study suggest that root, leaf and flower extracts have potent antioxidant activity against free radicals, which is comparable to that of Trolox.

3.9 Correlation between antiradical, anticancer and antibacterial activities and phenolic and carotenoid content in extracts

Many studies were undertaken to show the influence of phenolic and carotenoid components on the biological activity of plant extracts [24,30]. Likewise, our study was to define the relationship between the biological activity and chemical composition of the extracts. For this purpose, coefficients of correlation were calculated between amounts of determined compounds and percentage of dead cells in cytotoxic tests, MIC in antimicrobial study and IC_{50} values in antiradical ability determination, respectively (Table 4). This is the first attempt to analyze in detail the correlations between the activity and chemical composition of investigated extracts.

Carotenoids were not found to affect the antibacterial, antiproliferative and antiradical activity of extracts; considerable correlations were, however, observed for the majority of phenolic compounds.

	IC_{50}	AE	TE	QE	GAE
D Flos	0.27	3.67	1.47	3.89	8.26
D Fol	0.21	4.76	1.14	3.00	5.25
D P	1.27	0.79	6.87	18.15	31.77
D N	1.79	0.56	9.65	25.51	44.65
D Rx	0.22	4.64	1.16	3.08	5.38
T Flos	0.24	4.15	1.30	3.45	6.03
T Fol	0.22	4.48	1.21	3.19	5.58
T P	1.79	0.56	9.68	25.58	44.76
T N	0.62	1.61	3.36	8.88	15.54
T Rx	0.19	5.35	1.01	2.67	4.67

Table 3. Antiradical activity of *Rosa rugosa* Thunb. teas and tinctures. IC50 expressed as mg of dry extract per mg $DPPH^{\bullet}$, mean values of three replications; AE – antiradical efficiency ($1/IC_{50}$); TE - Trolox equivalent; QE - quercetin equivalent; GAE – gallic acid equivalent. Equivalents were calculated by dividing extract IC_{50} by standard IC_{50}. For Trolox IC_{50}=0.185 mg mg^{-1} $DPPH^{\bullet}$; for quercetin IC_{50}=0.07 mg mg^{-1} $DPPH^{\bullet}$; for gallic acid IC50=0.033 mg mg^{-1} $DPPH^{\bullet}$. Other abbreviations as in Table 1.

The correlation coefficients between phenolic content and anticancer activity were determined in two ways. The first analyzed group contained all preparations from all plant parts, whereas the second one contained extracts prepared from parts which were found to be abundant in phenolic compounds, *i.e.* all parts except nuts. The data obtained demonstrate a considerable impact of polyphenols on the anticancer activity of the extracts (particularly ethanolic ones). Numerous studies have reported on the antiproliferative activity of flavonoids and tannins [30]. A high cytotoxic activity of

Variables	Phenolic compounds	r Teas	r Tinctures
	Antimicrobial activity		
S. epidermidis	Phenolics	-0.465	-0.118
	Flavonoids	-0.780	-0.719
	o-dihydroxyphenols	-0.421	-0.445
	Tanins	0.357	-
S. aureus	Phenolics	-0.465	-0.260
	Flavonoids	-0.780	-0.750
	o-dihydroxyphenols	-0.421	-0.654
	Tanins	0.357	-
B. subtilis	Phenolics	nd	0.248
	Flavonoids	nd	-0.367
	o-dihydroxyphenols	nd	0.198
	Tanins	nd	-
M. luteus	Phenolics	nd	-0.653
	Flavonoids	nd	-0.975*
	o-dihydroxyphenols	nd	-0.697
	Tanins	nd	-
E. coli	Phenolics	-0.151	-0.704
	Flavonoids	-0.398	-0.924*
	o-dihydroxyphenols	-0.301	-0.861*
	Tanins	0.202	-
K. pneumoniae	Phenolics	-0.151	-0.653
	Flavonoids	-0.398	-0.950*
	o-dihydroxyphenols	-0.301	-0.775
	Tanins	0.202	-
P. aeruginosa	Phenolics	-0.151	-0.496
	Flavonoids	-0.398	-0.551
	o-dihydroxyphenols	-0.301	-0.832*
	Tanins	0.202	-
P. mirabilis	Phenolics	-0.151	-0.233
	Flavonoids	-0.398	-0.587
	o-dihydroxyphenols	-0.301	-0.217
	Tanins	0.202	-
	Antiradical DPPH• assay		
IC 50	Phenolics	-0.879*	-0.136
	Flavonoids	-0.969*	-0.607
	o-dihydroxyphenols	-0.964*	-0.668
	Tanins	-0.564	-
	Cytotoxic activity		
A549	Phenolics	0.465	-0.365
	Flavonoids	0.780	0.283
	o-dihydroxyphenols	0.421	0.384
	Tanins	-0.279	-
A549 without nuts	Phenolics	0.294	0.988*
	Flavonoids	0.785	0.993*
	o-dihydroxyphenols	0.154	0.969*
	Tanins	-0.661	-
TOV-112D	Phenolics	0.883*	-0.035
	Flavonoids	0.989*	0.589
	o-dihydroxyphenols	0.911*	0.672
	Tanins	0.461	-
TOV-112D without nuts	Phenolics	0.771	0.978*
	Flavonoids	0.979*	0.998*
	o-dihydroxyphenols	0.824*	0.980*
	Tanins	0.115	-

Table 4. Pearson's correlation coefficients between biological activities and concentrations of phenolic compounds in different kinds of rose galenic preparations. Statistically significant values were marked with an asterisk. Abbreviations: "nd" - not determined, "-" - not detected.

nuts (with low amounts of phenolics) probably depends on another group of compounds; hence further analysis of individual components is required.

According to the data presented in Tables 2 and 4, the antibacterial properties of rose galenic preparations against most of the bacterial strains studied were strongly influenced by flavonoids, except for tinctures where the presence of o-dihydroxyphenols was correlated with antipseudomonal potential. It is worth stressing, however, that synergistic and antagonistic effects between some components may affect the observed antimicrobial activity of plant extracts. In addition to the main components, minor components may also have antimicrobial properties.

Some previous studies report that antioxidant activity does not necessarily correlate with high amounts of phenolics [31]. On the other hand, there are numerous data demonstrating a strong relationship between high phenolic content and antioxidant activity of plant material [5,9,10]. Thus, we attempted to investigate these relationships in our extracts. It was demonstrated that the extent of the antioxidant activity depended on the phenolic group and type of extract. The correlation between IC_{50} and total phenolic content was found to be extremely high in teas, which is in accordance with the results reported in previous studies [24]. For teas, the values of the Pearson coefficient ranged from -0.564 to -0.969 for tannins and flavonoids, respectively. The correlations between phenols and antiradical activity of tinctures were surprisingly low (r from -0.136 to -0.668). This is likely to be related to the more complex chemical composition of tinctures compared to teas, which may result in superimposed interactions between the components of extracts (especially antagonistic ones). Moreover, non-phenolic compounds may significantly influence the antiradical activity of tinctures. To explain these relationships, we have decided to analyze comprehensively the composition of these extracts using the modern chromatographic (LC MS) method in our further studies.

Reports of other researchers have revealed the presence of flavonoids (including quercetin derivatives), phenolic acids (e.g. gallic acid, ellagic acid), o-dihydroxyphenols (e.g. caffeic acid) and hydrolysable tannins in various *R. rugosa* parts [1,8,12,32]. These compounds possess proven cytotoxic, antimicrobial and antioxidant activity [8,12,30]. Taking into consideration the types of extraction used in our study, these compounds should also be present in the tested samples.

Although some reports demonstrate that *R. rugosa* is a potential source of biologically active substances, the plant still seems to be virtually unknown, underestimated and underused. Our study confirms that its galenic preparations could be a rich source of natural radical scavengers and other beneficial substances which play an important role in the everyday healthy diet.

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