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INFLUENCE OF DIFFERENT EXTRACTION PROCEDURES ON THE ANTIRADICAL ACTIVITY AND PHENOLIC PROFILE OF *ROSA RUGOSA* PETALS

MARTA OLECH and RENATA NOWAK*

Department of Pharmaceutical Botany, Medical University of Lublin,
1 Chodźki St., 20-093 Lublin, Poland

Abstract: The present study was designed to develop conditions for the optimal extraction of antiradical ingredients of rugosa rose petals. The effect of temperature (22–140°C), solvent and extraction type on the phenolics extraction and antiradical activity was studied. In order to obtain extracts from rugosa rose petals with the highest activity and phenolic content, the use of mixture of polar organic solvents (acetone, in particular) with water is recommended.

Keywords: *Rosa rugosa*, antiradical, extraction conditions, DPPH, accelerated solvent extraction, phenolic profile

Antioxidants belong to the most desirable plant components and their application for food, cosmetic and medicinal purposes is becoming increasingly popular. Since oxidative stress accompanies the emergence of many diseases, e.g., neurodegenerative and cardiovascular disorders, cancers or arthritis (1), plants are expected to provide antioxidants of valuable medicinal properties.

Rugosa rose (*Rosa rugosa* Thunb.) is a rose cultivar grown mostly for its hips and flowers. Our previous research (2, 3) as well as the reports of other researchers have revealed the high antiradical activity of its petals (4, 5). Recent studies on natural compounds for medicine and cosmetics have drawn much attention to the effective extraction of the desired active ingredients of natural products. Therefore, knowing the activity of rugosa rose petals, it appears worthwhile to investigate which extraction method should be used in order to obtain the maximum amount of antiradical substances from this widely used plant.

Since antiradical activity is particularly related to the presence of phenolics (3, 6), it was decided to examine an impact of polar solvents on the extraction capacity of phenolic antioxidants and activity of extracts. To our knowledge, the influence of different extraction procedures of *R. rugosa* on its antiradical activity and phenolic content has not been studied yet.

EXPERIMENTAL

Chemicals and apparatus

Standards of gallic acid and Trolox (a water-soluble vitamin E analog) and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH·) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Ethanol, methanol and acetone were obtained from Polish Reagents – POCH (Gliwice, Poland). All chemicals were of analytical purity grade.

Accelerated solvent extractions were performed in the ASE 150 extractor from Dionex Corporation (Sunnyvale, CA, USA) and lyophilization of all obtained extracts in the Free Zone 1 apparatus (Labconco, Kansas City, KS, USA). All colorimertic measurements were conducted on 96-well transparent microplates (Nunclon, Nunc, Roskilde, Denmark) using an Elisa Reader Infinite Pro 200F (Tecan Group Ltd., Männedorf, Switzerland).

Plant material and preparation of extracts

Flowers of *Rosa rugosa* Thunb. were collected in Elizówka (near Lublin, Poland) in June 2009. The botanical material was authenticated and a voucher specimen was deposited in the Department of Pharmaceutical Botany, Medical University of Lublin, Poland. The plant material was dried at ambient temperature and powdered according to the 6th European Pharmacopoeia (7).

* Corresponding author: e-mail: renata.nowak@umlub.pl

Methanolic extract (M)

Twenty-five grams of dried and pulverized rose petals were extracted four times (for 24 h each), at ambient temperature with 150 mL of 80% (v/v) aqueous methanol. During the last extraction, a sonication step at 50°C (30 min) was applied in order to achieve exhaustive extraction. Extracts were combined, filtered and evaporated to dryness under vacuum. The residue was weighted and redissolved in 80% (v/v) methanol to obtain stock methanolic solutions (M) at a concentration of 100 mg/mL.

Acetonic extract (GA)

To obtain the acetonic extract, the procedure described by Naczk et al. was used (8). Briefly, pulverized plant material was extracted three times with 70% (v/v) acetone at a sample-to-solvent ratio of 15:100 (w/v) in a shaking bath at 50°C for 30 min. Extracts were combined, filtered and lyophilized. The residue was weighted and redissolved in 70% (v/v) acetone to obtain stock solutions (GA) at a concentration of 100 mg/mL.

Accelerated solvent extraction (ASE)

The extractions with four different water-ethanol proportions were performed using an accelerated solvent extraction system. Extractions were conducted at four different extraction temperatures (22, 45, 100 and 140°C) with water and three ethanol concentrations (50, 70 and 100%). The extraction procedure was as follows: 2 g of pulverized plant material was loaded into the extraction cell; the cell was filled with solvent up to a pressure of approximately 100 bars; extraction was performed for 10 min; the cell was rinsed with 60% cell volume using an extraction solvent; the solvent was purged from the cell with nitrogen and depressurization took place. The procedure was conducted three times for each sample. Extracts were combined and lyophilized. The residue was weighted and redissolved in the same solvent as used in extraction to obtain stock solutions at a concentration of 100 mg/mL. All extracts were prepared in duplicate and the obtained samples were marked with the symbols of solvent (i.e., H_2O – water extract, EtOH – ethanolic extract, 50% EtOH – extract obtained with 50% ethanol, 70% EtOH – extract obtained with 70% ethanol) and temperature (i.e., 22, 45, 100 and 140°C), e.g., 50% EtOH 100°C means the extract obtained by extraction with 50% ethanol at a temperature of 100°C.

Tea and tincture

Infusion (tea) and ethanolic tincture were prepared according to the monographs of the Polish Pharmacopoeia 6th edition (9). For tea preparation, 100 mL of boiling water was poured over 2 g of pulverized plant material and left to cool; tea was filtered and filled up with distilled water to the volume of 100 mL.

Tincture was prepared from 10 g of pulverized plant material extracted with 50 g of 70° ethanol and left in a closed glass container for seven days at ambient temperature (the mixture was stirred occasionally three times a day). After this time, tincture was filtered and filled up with 70° ethanol to the measured volume. All extracts were prepared in duplicate and the obtained samples were marked with the following symbols: D – infusion (tea), T – tincture.

DPPH· radical scavenging

The scavenging effect of galenic preparations was monitored as previously described with some modifications (2). Aliquots of 190 µL of a freshly-prepared 2,2-diphenyl-1-picrylhydrazyl (DPPH·) colored solution in methanol (0.07 mg/mL) 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.

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

$$\% \text{ Inhibition} = [(A_B - A_A) / A_B] \times 100$$

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) and gallic acid (GAE) based on their IC_{50} values. Moreover, the antiradical efficiency ($AE = 1/IC_{50}$) was calculated.

Total phenolic content (TPC)

Total phenolic content was assayed on microplates by the modified Folin-Ciocalteau method (10). Briefly, 20 µL of the examined extract was added to 20 µL of diluted Folin-Ciocalteu reagent (with water 1:4, v/v) followed by addition of 160 µL of sodium carbonate (75 g/L). The absorbance was measured at 680 nm after 20 min using an Elisa reader with the solution containing water instead of the Folin-Ciocalteau reagent as a

blank. The results were expressed as mg of gallic acid per mg of dry extract.

Total flavonoid content (TFC)

The flavonoid content was determined according to the method described by Lamaison and Carret (11) with some modifications; 20 µL of sample was mixed on a microplate with 60 µL of 96% (v/v) ethanol, 4 µL of 10% (v/v) $AlCl_3$ water solution, 4 µL of 1M sodium acetate and filled with water to the volume of 200 µL. Absorbance was estimated at 430 nm after 30 min with the solution containing water instead of sample as a blank. The total flavonoid content was expressed as mg of quercetin per 1 mg of dry extract.

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.

RESULTS AND DISCUSSION

Twenty extracts prepared from rugosa rose petals were screened for their antiradical activity. Two of them were prepared by classical extraction methods, 16 were obtained using accelerated solvent extraction and two were typical galenic preparations (tea and tincture). The influence of solvent type and extraction temperature on antiradical efficacy was investigated.

Previous studies have revealed the presence of different groups of polyphenols in rugosa rose petals. These included flavonoids (i.e., quercetin and kaempferol derivatives), hydrolyzable and condensed tannins (e.g., rugosin A and D, tellimagrandin I and II) and phenolic acids (4, 5, 12, 13).

Since our previous research demonstrated a considerable impact of phenolics on extract antiradical activity (2, 3), the present study was focused on

Table 1. Antiradical activity of *Rosa rugosa* Thunb. extracts. IC_{50} expressed as mg of dry extract per mg $DPPH^{\cdot}$, mean values of three replications; AE – antiradical efficiency ($1/IC_{50}$); TE – Trolox 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 $DPPH^{\cdot}$; for gallic acid IC_{50} = 0.033 mg/mg $DPPH^{\cdot}$; M – methanolic extract; GA – acetonic extract; D (*decoctum*) – tea; T – tincture.

Sample	IC_{50}	AE	GAE	TE
H_2O 22°C	0.73	1.38	24.20	3.82
H_2O 45°C	0.71	1.41	23.72	3.75
H_2O 100°C	0.85	1.17	28.48	4.50
H_2O 140°C	0.59	1.70	19.60	3.09
50EtOH 22°C	0.21	4.75	7.01	1.11
50EtOH 45°C	0.31	3.21	10.38	1.64
50EtOH 100°C	0.39	2.58	12.93	2.04
50EtOH 140°C	0.48	2.09	15.97	2.52
70EtOH 22°C	0.44	2.26	14.74	2.33
70EtOH 45°C	0.40	2.53	13.19	2.08
70EtOH 100°C	0.30	3.33	10.00	1.58
70EtOH 140°C	0.29	3.38	9.87	1.56
EtOH 22°C	1.08	0.93	35.88	5.67
EtOH 45°C	1.12	0.89	37.38	5.90
EtOH 100°C	0.79	1.27	26.29	4.15
EtOH 140°C	0.94	1.07	31.19	4.93
M	0.28	3.56	9.38	1.48
GA	0.19	5.28	6.32	1.00
T	0.24	4.15	8.04	1.27
D	0.27	4.48	7.44	1.17

the antioxidant extraction capacity of polar solvents. The activities of water, ethanolic, methanolic and acetonic extracts were compared. Water and ethanol (70%, in particular) are commonly used for extraction of plant material in household settings and in herbal medicine (e.g., teas and tinctures); 70% acetone was also chosen because of its high ability to elute phenolics (8, 14).

As accelerated solvent extraction has been used for extraction of various biological materials, our aim was to examine the usefulness of this method for extraction of rose antioxidants. To our knowledge, pressurized solvent extraction of rugosa rose petals has not been reported to date. The study was also designed to check the influence of temperature during accelerated solvent extraction on antiradical activity and possible degradation of heat-sensitive bioactive compounds present in rose petals. The chosen temperature range was wide, from 22°C – close to room temperature, through 45°C, to higher temperatures 100°C and 140°C.

As shown in Table 1, all examined extracts demonstrated high antiradical potential, with the highest activity of the acetonic one (IC_{50} = 0.19 mg/mg DPPH·). In general, the best solvents for antiradicals were mixtures of water and acetone, ethanol or methanol. Extracts made with the use of these solvents showed high activity with IC_{50} values ranging from 0.19 to 0.48 mg/mg DPPH·. In water-ethanolic pressurized solvent extracted samples, the temperature did not affect significantly their extraction efficiency.

Lower activities were observed for pressurized solvent extracted water samples with IC_{50} values ranging from 0.59 to 0.85 mg/mg DPPH· for H_2O 140°C and H_2O 100°C, respectively. The extraction efficiency was slightly increased with increasing temperature, with the exception of the extract H_2O 100°C, which showed the highest IC_{50} value in the group.

Antiradical activity of extracts obtained with 99.8% ethanol was considerably decreased. Moreover, at lower temperatures, ethanolic extracts

Table 2. Extraction efficiencies, total phenolic (TPC) and flavonoid (TFC) contents, antiradical efficiencies (AE = $1/IC_{50}$) of different extracts prepared from rugosa rose petals. Mean values of three replications; M – methanolic extract; GA – acetonic extract; D (*decoctum*) – tea; T – tincture.

Sample	TPC µg of gallic acid/ mg of dry extract	TFC µg of quercetin/ mg of dry extract	Efficiency of extraction [g of dry extract obtained from 1 g of raw material]	TPC mg/g of raw material	AE
H_2O 22°C	96.91 ± 2.85	3.36 ± 0.11	0.575	55.72	1.38
H_2O 45°C	113.48 ± 3.18	4.54 ± 0.48	0.604	68.54	1.41
H_2O 100°C	54.34 ± 1.32	1.75 ± 0.04	0.875	47.51	1.17
H_2O 140°C	86.61 ± 1.88	2.68 ± 0.25	0.776	67.21	1.70
50EtOH 22°C	234.98 ± 3.76	3.79 ± 0.10	0.475	111.62	4.75
50EtOH 45°C	200.19 ± 5.27	3.17 ± 0.04	0.510	102.10	3.21
50EtOH 100°C	140.12 ± 1.80	2.79 ± 0.09	0.838	117.42	2.58
50EtOH 140°C	129.24 ± 1.86	3.21 ± 0.16	0.850	109.85	2.09
70EtOH 22°C	139.60 ± 4.51	1.94 ± 0.02	0.531	74.13	2.26
70EtOH 45°C	131.85 ± 5.11	2.41 ± 0.08	0.595	78.45	2.53
70EtOH 100°C	167.70 ± 5.86	3.57 ± 0.12	0.656	110.01	3.33
70EtOH 140°C	183.03 ± 5.54	5.05 ± 0.04	0.784	143.50	3.38
EtOH 22°C	62.70 ± 0.92	1.46 ± 0.03	0.211	13.23	0.93
EtOH 45°C	56.81 ± 1.31	1.38 ± 0.05	0.425	24.14	0.89
EtOH 100°C	73.75 ± 1.38	1.35 ± 0.06	0.611	45.06	1.27
EtOH 140°C	109.30 ± 2.26	2.08 ± 0.09	0.699	76.40	1.07
M	171.81 ± 4.96	2.62 ± 0.02	0.520	89.34	3.56
GA	256.74 ± 4.92	1.97 ± 0.05	0.509	130.68	5.28
T	194.39 ± 2.16	1.85 ± 0.18	0.197	38.29	4.15
D	161.63 ± 2.36	5.83 ± 0.25	0.600	96.98	4.48

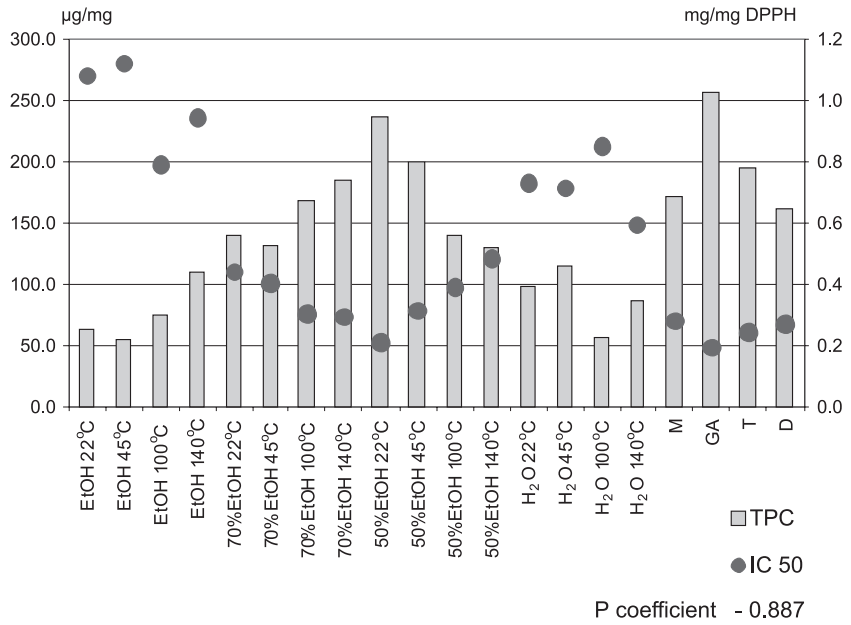

Figure 1. Total phenolic contents (TPC) expressed in µg of gallic acid/mg of dry extract, IC_{50} values expressed in mg/mg DPPH• and Pearson's correlation coefficient between activities and concentrations of phenolics in different rose extracts

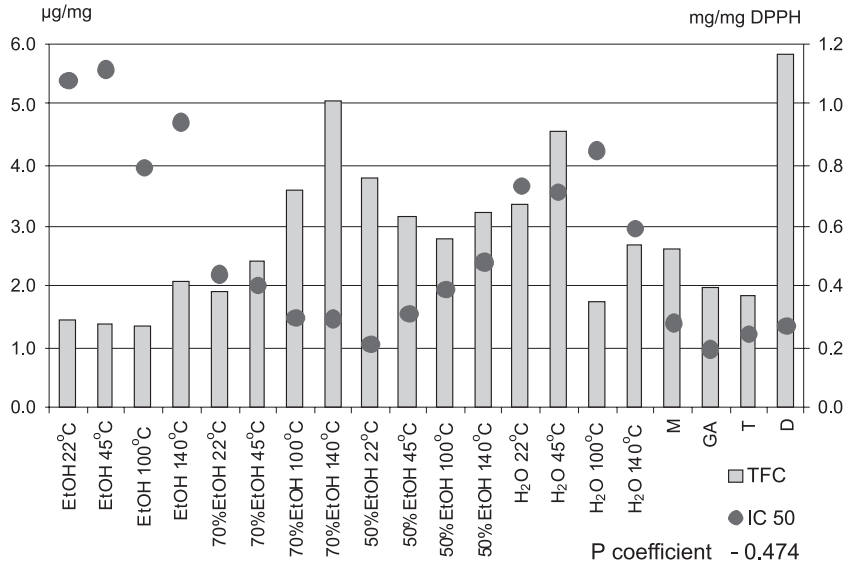

Figure 2. Total flavonoid contents (TFC), IC_{50} values and Pearson's correlation coefficient between activities and concentrations of flavonoids in different rose extracts. TFC is expressed in µg of quercetin/mg of dry extract, IC_{50} values are expressed in mg/mg DPPH•

with the weakest activity were obtained (IC_{50} values 1.08 to 1.12 mg/mg DPPH· for EtOH 22°C and EtOH 45°C, respectively). At higher temperatures, the increased activity was observed (IC_{50} was 0.79 to 0.94 mg/mg DPPH· for EtOH 100°C and EtOH 140°C, respectively). Although the mixture of solvents enables the most efficient extraction, it is the presence of water which is essential for extraction of antioxidants; the amount of water added is less important, whether at 20 or 50%. Therefore, it can be concluded that a significant part of petal activity depends on water-soluble compounds.

Since polar solvents were used in the study, quantities of phenols and flavonoids extracted from the raw material using different methods were examined. For this purpose, total phenolic contents (TPC) were assayed by the modified Folin-Ciocalteau method (10) and total flavonoid contents (TFC) were determined according to the Lamaison and Carret method (11). As shown in Table 2, significant differences in total phenolic content were observed. TPC varied from 54.34 to 256.74 μg/mg of dry extract for EtOH 45°C and GA, respectively (the fivefold difference). The best solvents for phenolic extraction were mixtures of water and acetone, ethanol or methanol. Extracts made with the use of these solvents contained large amounts of phenolics, ranging from 129.24 to 256.74 μg/mg. The use of solvents without water considerably reduced the degree of phenolic extraction. In the case of pressurized solvent extracted samples, the temperature affected the samples differently, depending on the solvent used. In most cases, it did not result in major changes in extraction efficiency, except for samples extracted with 50% ethanol, where lower temperatures were found to be more appropriate.

The TFC varied from 1.351 to 5.053 μg of quercetin per mg of dry extract for EtOH 100°C and 70EtOH 140°C, respectively (Table 2); and again, the extraction with 99.8% ethanol resulted in solutions with the lowest concentrations of the substances examined.

As shown in Table 2, the higher the extraction temperature, the higher the extract mass is. However, this does not mean that the amount of extracted antioxidants increases. In "H_2O" and „50EtOH" extracts, the opposite is observed. Most likely, only the content of ballast components, which have no impact on antioxidant activity, grows.

It is noteworthy that the extract presenting the highest antiradical activity (GA) contained the highest amount of polyphenols, while the weakest one (EtOH 45°C) demonstrated almost the lowest TPC.

To define in detail the relationship between the biological activity and extract phenolic profile, the TPC/TFC and IC_{50} values were collated in Figures 1 and 2 and coefficients of correlation were calculated. It was demonstrated that the extent of antioxidant activity depended on the concentration of phenolics. As shown in Figures 1 and 2, the correlation between IC_{50} and total phenolic content was found to be extremely high (R2 – 0.887), which is in accordance with the results reported in previous studies (2, 15). In the case of flavonoids, their impact on the extract activity is inconclusive (R2 – 0.473). Although an increase in antiradical potential with increasing concentration of flavonoids is observed, their effect on activity of rose petals is not essential. Their activity may be simply overshadowed by the activity of polyphenols present in much higher concentrations.

Activities and composition of methanolic, acetonic and accelerated solvent extraction samples were compared with antiradical potential of two galenic preparations obtained from the plant material. The conditions of accelerated solvent extraction as well as GA and M extractions were designed to achieve complete extraction. Extraction of galenic preparations was carried out according to the monographs described in the Polish Pharmacopoeia (9). For tea preparations, plant material was poured with boiling water only once, left to cool and filtered; therefore, an extraction time was limited. In the case of tinctures, material had indeed longer contact with the solvent, but permanently with its one portion and extraction was carried out only at room temperature. Thus, it should be expected that these conditions are not sufficient to extract all active compounds. However, phenolic and flavonoid content of teas and tinctures was comparable to that of M, GA and accelerated solvent extraction samples. Moreover, the galenic preparations tested were also found to be sufficient for extraction of antioxidants.

CONCLUSION

In order to obtain extracts from rugosa rose petals with the highest activity and phenolic content, the use of mixture of polar organic solvents (acetone, in particular) with water is recommended. The method chosen (traditional or ASE) is less important. However, the extraction temperature when using aforementioned mixtures should not exceed 100°C, since large amounts of ballast compounds are then extracted.

Although the use of accelerated solvent extraction is not necessary to obtain good quality extracts, this rapid method can be successfully applied in the production of rose extracts as ASE offers a lower cost per sample than other techniques and reduces solvent consumption.

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