

Phytobiological Characteristics of *Rosa hemisphaerica* Herrm. Extract

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Abstract

Background: Roses are one of the most important groups of ornamental plants referred to as the queen of flowers and their fruits and flowers are used in a wide variety of food, nutritional products and different traditional medicines. A large diversity is expected to be found in Iranian Damask rose landraces with different biological properties.

Objective: The antibacterial, antioxidant and cytotoxicity of methanolic and aqueous extracts of *Rosa hemisphaerica* Herrm. were studied.

Methods: The agar disc diffusion method was employed for the determination of antimicrobial activities. Total phenol contents of both extracts were estimated. Antioxidative properties of the extracts were determined by bleaching of beta carotene or 2, 20-diphenylpicrylhydrazyl (DPPH). The Ferric-Reducing Antioxidant Power (FRAP) was expressed as gallic acid equivalents. Cytotoxicity of the extracts were tested using Hela and human healthy peripheral blood cells.

Results: of various microorganisms tested, only *S. aureus* was found susceptible. The total phenol contents of the methanolic and aqueous extracts were 138.33 ± 11.37 and 129.67 ± 4.51 μ g Gallic acid equivalent/mg sample respectively. DPPH scavenging and lipid peroxidation inhibition effects were higher than those of the synthetic antioxidants. Ferric-reducing antioxidant power (FRAP) of the methanolic and aqueous extracts were determined as 96.134 ± 4.25 mg/g and 98.63 ± 2.51 mg/g respectively. The 50% cytotoxic concentrations were found to be 0.327 mg/ml 0.177 mg/ml for Hela cells and human lymphocytes respectively.

Conclusion: It can be concluded from the above results that *R. hemisphaerica* extract exhibited antimicrobial activity only against *S. aureus*. The extracts provided better antioxidative activity as compared with synthetic antioxidants. The extracts were toxic to the Hela cells as well as human lymphocytes. Hence, the *R. hemisphaerica* extract may be exploited as a natural antioxidant and health promoting agent.

Keywords: Rose, *Rosa hemisphaerica* Herrm., Antimicrobial, Antioxidant, Cytotoxicity

Introduction

Members of the Rosaceae family have long been used for food and medicinal purposes. *Rosa damascena* is also cultivated for its medicinal properties and this aspect is steadily increasing in the world. Because of the low oil content in *Rosa genus* and the lack of natural and synthetic substitutes, rose oil is one of the most expensive essential oils in the world markets [1]. Rose hips are very rich in phenolics [2]. Rose hips are well known for their efficacy in strengthening the body's defense against infection, and particularly the common cold. The fruits, leaves and even roots are boiled in water and used as diuretics and as ingredients in common cold remedies [3]. Rose hips are also well known to have the highest vitamin C content (300 – 4000 mg/100 g) among fruits and vegetables. In addition, rose hips contain other vitamins and minerals, carotenoids, tocopherol, bioflavonoids, fruit acids, tannins, pectin, sugars, organic acids, amino acids and essential oils [4, 5]. Antibacterial activity of fresh and spent *Rosa damascena* flower extracts was determined by the agar diffusion method against 15 species of bacteria: *Aeromonas hydrophila*, *Bacillus cereus*, *Enterobacter aerogenes*, *Enterococcus faecalis*, *Escherichia coli*, *Escherichia coli* O157:H7, *Klebsiella pneumoniae*, *Mycobacterium smegmatis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Salmonella enteritidis*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Yersinia enterocolitica*. Statistical differences among bacteria were significant at $p < 0.05$. Both extracts were effective against all the bacteria except *E. coli* O157:H7, although the fresh extract was more effective than the spent extract. Fresh and spent extracts showed the strongest effects against *S. enteritidis* and *M.*

smegmatis, respectively [6]. Antimicrobial activity of *Rosa damascena* was recorded only to *Staphylococcus aureus* [7]. Total aerobic mesophilic bacteria, Enterococci, Enterobacteriaceae and *Staphylococcus aureus* counts were decreased in the dried rose dreg group as an alternative litter material for broiler performance and microbiological characteristics of litter at 42 days. Dried rose dreg can be used as an alternative litter material to control microbial characteristics without a negative effect on broiler performance [8]. The essential oil of *R. damascena* at the Minimum Bactericidal Concentration (MBC) of 1386.5 µg/ ml was suggested to be a useful natural bactericide for the control of the fire blight pathogen, *Erwinia amylovora* [9]. *R. damascena* is a potent antioxidant that has many therapeutic uses in addition to its perfuming effects. Supplementing *Drosophila* with rose extract resulted in a statistically significant decrease in mortality rate in male and female flies. Moreover, the observed anti-aging effects were not associated with common confounds of anti-aging properties, such as a decrease in fecundity or metabolic rate [10]. The antioxidant [11], antispasmodic, cardiovascular preventive, antibacterial and skin protective effects of extracts from this plant have been reported [12, 13]. *R. damascena* first grew wild and it is still self-growing in Caucasus, Syria, Morocco and Andalusia. Iran has also been mentioned as one of its origins [14]. In Iran, cultivation and consumption of *R. damascena* has a long history. It is believed that the crude distillation of roses for the oil was originated from Persia in the late 7th century A.D., and spread to the provinces of Ottoman Empire later in 14th century. Iran was the main producer of rose oil until the 16th century and exported it to all

around the world [15]. Therefore, a large diversity is expected to be found in Iranian Damask rose landraces. These landraces, from different geographical areas, could have evolved different gene complexes favoring adaptation to local environmental conditions. However, no report till date was available on the characterization of phenolic constituents in wild growing *R. damascena* until recently reported an RP-HPLC method for the determination of polyphenols in *R. damascena*, *R. bourboniana* and *R. brunonii* [16]. Roses are one of the most important groups of ornamental plants referred to as the queen of flowers and their fruits and flowers are used in a wide variety of food, nutritional products and different traditional medicines [17]. Over 150 rose species and more than 2000 cultivars have been registered [18]. A wide diversity of phytochemicals exists within *Rosa* genera [2;19]. It is well established that genotype or species may have a profound influence on the content of bioactive compounds in small fruits [20]. With a view to the large diversity of Rosaceae family this study was aimed to determine the health promoting potentials of *Rosa hemisphaerica* Herrm. extracts grown in Iran. Thus, herein we report the assessment of antimicrobial, antioxidant capacities, total phenolics, acute and cytotoxicity of *Rosa hemisphaerica* Herrm. extracts.

Materials and methods

Equipments and Chemicals

The major equipments used were, UV-2501PC spectrophotometer, ELISA reader and routine microbiology laboratory equipments. Microbial and cell culture media and laboratory reagents were from Merck, Germany. Other chemicals were of analytical grade.

Preparation of extracts

Rosa hemisphaerica collected from the natural rose gardens of Kashan city of Iran were shadow dried. The dried flowers were ground finely. Aqueous extract was prepared by adding 100 g of the powder to 500 ml of boiling water for 30 minutes. After filtration, the extract was lyophilized with a freeze-dryer and stored at 4°C. 500 ml of methanol was used for methanolic extraction at room temperature for 3 h. After extraction, the mixture was filtered and the residue was re-extracted with 500 ml of fresh methanol overnight. The combined methanolic solution was centrifuged at 12,000g for 10 min. The extracts were distilled under vacuum at 40 °C, dried in lyophilizer and stored at 4 °C until use. The methanolic extract was reconstituted in dimethyl sulfoxide (DMSO) to a concentration of 400 mg/ml for subsequent experimentation.

Microbial strain and growth media

E. coli (ATCC 25922), *S. aureus* (ATCC 25923), *Streptococcus faecalis* (PTCC 33186), *Pseudomonas aeruginosa* (ATCC 8830) and *Klebsiella pneumoniae* (ATCC 13883) were employed in the study. Bacterial suspensions were made in Brain Heart Infusion (BHI) broth to a concentration of approximately 10⁸ cfu/ml. Subsequent dilutions were made from the above suspension, which were then used in the tests.

Extract sterility test

In order to ensure sterility of the extracts, geometric dilutions ranging from 0.04 to 80 mg/ml of the extracts, were prepared in a 96-well microtitre plate, including one growth control (BHI+DMSO) and one sterility control (BHI+DMSO+test extract). Plates were

incubated under normal atmospheric conditions, at 37°C for 24 h. The contaminating bacterial growth, if at all, was indicated by the presence of a white “pellet” on the well bottom. The extracts were filter sterilized, as and when needed, using 0.45µ sterile filter.

Disc diffusion method

The agar disc diffusion method was employed for the determination of antimicrobial activities of the extracts in question. Briefly, 0.1 ml from 108 CFU/mL bacterial suspension was spread on the Mueller Hinton Agar (MHA) plates. The agar was bored with a sterile borer (6 mm in diameter). 50 µl of the 20 mg/ml and 10mg/ml dilutions of each extract were placed in the wells of the inoculated plates. The plates were allowed to stand for 1 hour at room temperature, then at 4°C for 2 h. The plates were then incubated at 37°C for 24 h. The diameters of the inhibition zones were measured in millimeters. All tests were performed in triplicate.

Total phenolic content assay

Total phenol content was estimated as gallic acid equivalents (GAE; mg gallic acid/g extract) as described earlier [21]. In brief, a 100 µl aliquot of dissolved extract was transferred to a volumetric flask, containing 46.0 ml distilled H₂O, to which was subsequently added 1 ml Folin–Ciocalteu reagent. After 3 mins, 3 ml of 2% Na₂CO₃ was added. After 2 h of incubation at 25°C, the absorbance was measured at 760 nm. Gallic acid (Sigma Co., 0.2–1 mg/ml gallic acid) was used as the standard for the calibration curve, and the total phenolic contents were expressed as mg gallic acid equivalents per gram of tested extracts ($Y=0.001x + 0.0079$; $r^2 = 0.9967$).

DPPH Radical scavenging capacity of the extracts

The hydrogen atom or electron donation abilities of the corresponding extracts and some pure compounds were measured from the bleaching of the purple-colored methanol solution of 2, 20-diphenylpicrylhydrazyl (DPPH). Two ml of different dilutions of the extract in methanol were added to two ml of a 0.0094% methanol solution of DPPH. Trolox (1 mM) (Sigma-Aldrich), a stable antioxidant, was used as a synthetic reference. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of free radical by DPPH in percent (I%) was calculated in following way:

$$I\% = (A_{\text{blank}} - A_{\text{sample}}/A_{\text{blank}}) \times 100;$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. Tests were carried out in triplicate.

Lipid peroxidation inhibition activity

Lipid peroxidation inhibition activity was determined using the β-carotene bleaching assay. Approximately 5 mg of β-carotene (type I synthetic, Sigma–Aldrich) was dissolved in 10 ml of chloroform. The carotene-chloroform solution, 1.5 ml, was pipetted into a boiling flask containing 33.82 mg linoleic acid (Sigma–Aldrich) and 300 mg Tween 40 (Sigma–Aldrich). Chloroform was removed using a rotary evaporator at 40°C for 5 min and, to the residue, 150 ml of distilled water were added, slowly with vigorous agitation, to form an emulsion. 2.5 ml of the emulsion were added to a tube containing 350 µl of the test extract dilutions and the absorbance was immediately measured at 470 nm against a blank, consisting of an emulsion without β-carotene. The tubes were placed in a water

bath at 50°C and the oxidation of the emulsion was monitored spectrophotometrically by measuring absorbance at 470 nm over 30, 60 and 90 minute periods. Control samples contained 350 µl of water instead of the test extract. Butylated hydroxy anisole (BHA) and butylated hydroxytoluene (BHT), stable antioxidants, were used as synthetic references. Lipid peroxidation inhibition activity was expressed as percent antioxidant activity AOA (%) and calculated as follows:

Bleaching rate (BR) of β -carotene = $\ln(A_{\text{initial}}/A_{\text{sample}})/\text{time (minutes)}$

AOA (%) = $1 - (BR_{\text{sample}}/BR_{\text{control}}) \times 100$

Where A_{initial} and A_{sample} are absorbance of emulsion before and after incubation period, and (BR_{sample} and BR_{control}) are bleaching rates of the sample and negative control respectively.

Ferric-reducing antioxidant power (FRAP) assay of the extract

The FRAP assay was carried out according to the procedure employed by Lim et al. [22]. One millilitre of the extract dilution was added to 2.5 ml of 0.2 M potassium phosphate buffer (pH 6.6) and 2.5 ml 1% potassium ferricyanide. The mixture was incubated for 20 min at 50 °C, after which 2.5 ml of 10% trichloroacetic acid was added. The mixture was then separated into aliquots of 2.5 ml and mixed with 2.5 ml of deionised water. Then, 0.5 ml of 0.1% (w/v) FeCl_3 were added to each tube and allowed to stand for 30 min. Absorbance for each tube was measured at 700 nm. The FRAP was expressed as gallic acid equivalents (GAE) in mg/g of samples used ($y = 16.66x + 0.003$; $r^2 = 0.999$).

Cytotoxicity assay

The human peripheral monocyte and human cervical carcinoma Hela cell line NCBI code No. 115 (ATCC number CCL-2) were

procured from Pasteur Institute, Tehran-Iran. The cells were grown in RPMI 1640 supplemented with 10% fetal calf serum, 1% (w/v) glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin. Cells were cultured in a humidified atmosphere at 37 °C in 5% CO_2 . Cytotoxicity was measured using a modified MTT assay. This assay detects the reduction of MTT [3-(4,5-dimethylthiazolyl)-2,5-diphenyltetrazolium bromide] by mitochondrial dehydrogenase, to blue formazan product, which reflects the normal functioning of mitochondrial and cell viability [23]. Briefly, the cells (5×10^4) were seeded in each well containing 100 µl of the RPMI medium supplemented with 10% FBS in a 96-well plate. After 24 h of adhesion, a serial of doubling dilution of the test extract was added to triplicate wells to the final concentration range of 5 – 0.1 mg/ml reaction well. The final concentration of ethanol in the culture medium was maintained at 0.5% (volume/volume) to avoid toxicity of the solvent [24]. After 2 days, 10 µl of MTT (5 mg/ml stock solution) were added and the plates were incubated for an additional 4 h. The medium was discarded and the formazan blue, which formed in the cells, were dissolved with 100 µl dimethyl sulphoxide (DMSO). The optical density was measured at 490 nm using a microplate ELISA reader. The cell survival curves were calculated from cells incubated in the presence of 0.5% ethanol. Cytotoxicity is expressed as the concentration of drug inhibiting cell growth by 50% (IC_{50}), ($y = 2154.3x + 40.22$; $r^2 = 0.974$). All tests and analyses were run in triplicate and mean values recorded.

Statistical analysis

All the experimental data are presented as mean \pm SEM of three individual samples. Data are presented as percentage of free radical scavenging/inhibition lipid peroxidation on

different concentration of *Rosa hemisphaerica* extract. IC₅₀ (the concentration required to scavenge 50% of free radicals) value was calculated from the dose-response curves. Antibacterial effect was measured in terms of zone of inhibition to an accuracy of 0.1 mm and the effect was calculated as a mean of triplicate tests. All of the statistical analyses were performed with the level of significant difference between compared data sets being set at $p < 0.05$.

Results

The antibacterial effect of *Rosa hemisphaerica* extracts were tested against some pathogens by agar diffusion and dilution methods. *S. aureus* was equally sensitive to both methanolic and aqueous extracts. *E. coli*, *S. faecalis*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were resistant (Table 1).

The total phenol contents (TPC) of the methanolic and aqueous extracts of *R. hemisphaerica* flower were determined to be 138.33 ± 11.37 and 129.67 ± 4.51 µg Gallic acid equivalent /mg sample (GAE/mg) respectively (Table 2).

Tables 2 - 4 show the antioxidant capacities of the rose extracts as assessed by different assay methods. *R. hemisphaerica* extracts exhibited a dose-dependent scavenging of DPPH radicals. Methanolic and aqueous extracts at 3.9 µg/ml and 5.6 µg/ml levels were sufficient to scavenge 50% of DPPH radicals respectively (Table 2). Lipid peroxidation inhibition activity of *Rosa hemisphaerica* extracts determined by β-carotene bleaching assay revealed statistically equal potency to the standard BHT and BHA (Table 3).

Ferric-reducing antioxidant power (FRAP) of the methanolic and aqueous extracts were determined as 96.134 ± 4.25 mg/g and 98.63 ± 2.51 mg/g respectively (Table 4).

The aqueous extract of *R. hemisphaerica* at 0.5 mg/ml concentration destructed Hela cells and human lymphocytes by 58.47% and 55.15% respectively (Table 5). The 50% cytotoxic concentrations were found to be 0.327mg/ml 0.177mg/ml for Hela cells and human lymphocytes respectively (Table 5).

Discussion

Antimicrobial activity

Susceptibility of *S. aureus* is consistent with those reported earlier [7, 8]. Both water and ethanolic extracts of *Rosa hemisphaerica* were effective on methicillin-resistant *Staphylococcus aureus* (MRSA). The resistance of *E. coli* in the present study confirms report of other investigators [6]. It is suggested that the phenolics compounds which are antioxidants are responsible for the anti bacterial activity [25].

Total phenolics content (TPC)

In other study the TPC of methanolic extracts of *R. damascene* flowers contained 145 ± 1.4 mg GAE/g [17]. The total phenolic contents of fresh and spent Rosa damascene flower extracts were reported as 276.02 ± 2.93 and 248.97 ± 2.96 mg GAE/g respectively [6] which is almost double amount of our findings. The higher phenolic acid levels in methanolic extracts could be due to extraction of both nonpolar and semipolar soluble phenolic acids.

Antioxidant activity

Many different methods have been established for evaluating the antioxidant capacity of certain biological samples, with such methods being classified, roughly, into one of two categories based upon the nature of the reaction that the method involved [26]. The methods involving an electron-transfer

Table 1- Determination of growth inhibition zone of *S. aureus* exposed to *Rosa hemisphaerica* extracts

	Mean Inhibition Zone (mm) 50µl (1 mg)/Well	Mean Inhibition Zone (mm) 50µl (0.5 mg)/Well
Methanolic extract	14.0±1.0	10.50±0.71
Aqueous extract	15.33±0.58	12.50±0.71

Table 2- Total phenolics of *Rosa hemisphaerica* extracts and Mean Inhibition of DPPH free radical (%)

Extracts and synthetic antioxidants	DPPH scavenging effect (%)	DPPH (IC ₅₀) (µg/ml)	Total phenolic content GAE µg Gallic acid/mg sample
Methanolic extract (1 mg/ml)	97.51 ± 0.068	3.9	138.33 ± 11.37
Aqueous extract (1 mg/ml)	96.85 ± 0.07	5.6	129.67 ± 4.51
BHT 1mM	35.9 ± 0.47	—	—
BHA 1mM	47.7 ± 0.47	—	—
Trolox 1mM	34.5 ± 0.4	—	—

reaction include the total phenolics assay using Folin – Ciocalteu reagent, the TEAC and the DPPH radical-scavenging assay.

The IC₅₀ value for the methanolic extract of *R. damascena* was reported relatively as low as 21.4 µg/ml [17]. The partially purified acetone fraction of *Rosa damascena* flower required for 50% inhibition of superoxide radical production, hydroxyl radical generation and lipid peroxide formation were 13.75, 135 and 410 µg/ml, respectively [11]. In the present study DPPH scavenging effect (%) of the extracts were significantly higher than those of the synthetic antioxidants (Table 2). Fresh and spent *Rosa damascena* flower extracts showed 74.51 ± 1.65 and $75.94 \pm 1.72\%$ antiradical activities at 100ppm [6]. Which are lower than those of our extracts. The DPPH radical scavenging is a sensitive antioxidant assay and is independent of substrate polarity [27]. DPPH is a stable free radical that can accept an electron or hydrogen radical to become a stable diamagnetic

molecule. A significant correlation was shown to exist between the phenolic content and with DPPH scavenging capacity for each spice [28]. Thus, owing to high content of polyphenols, rose extracts showed high antioxidant activities. These phenolic antioxidants play important role as bioactive principles in the rose flowers used as traditional medicines [17]. LPI activity is mainly attributed to the hydrophobic character of the antioxidant molecules but total phenolics content (TPC) measures both types of antioxidants, hydrophobic and hydrophilic [22]. The high antioxidant activity of the *R. damascena* extracts could be attributed to its high phenolic content.

Cytotoxicity

The IC₅₀ shows that cytotoxicity of the extract towards human tumor cell line is higher than that required for human healthy cells. These results indicate adverse side effects of the extract.

Table 3- Lipid peroxidation inhibition activity of *Rosa hemisphaerica* extracts determined by β -carotene bleaching assay at different time intervals

Antioxidant agents	30 minutes	60 minutes	90 minutes
<i>Rosa hemisphaerica</i> methanolic extract	49.72 \pm 3.26	79.01 \pm 2.91	82.43 \pm 2.61
<i>Rosa hemisphaerica</i> aqueous extract	48.57 \pm 3.33	78.30 \pm 2.89	81.51 \pm 2.74
BHT 1mM	54.84 \pm 2.55	78.54 \pm 2.46	81.15 \pm 2.35
BHA 1mM	54.93 \pm 2.65	78.49 \pm 2.43	81.48 \pm 2.50

Table 4- Ferric-Reducing Antioxidant Power (FRAP) of *Rosa hemisphaerica* extracts (Gallic acid equivalent (mg/g))

	Methanolic extract	Aqueous extract
1 mg/ml	96.47 \pm 0.43	100.65 \pm 0.36
0.5 mg/ml	47.87 \pm 0.49	49.73 \pm 0.47
0.25 mg/ml	22.74 \pm 0.78	24.86 \pm 0.75
0.1 mg/ml	10.14 \pm 0.48	9.50 \pm 0.36
Mean GAE (mg/g)	96.134 \pm 4.25	98.63 \pm 2.51

Table 5- Cytotoxicity assay of *Rosa hemisphaerica* aqueous extract

Extract Dilutions (mg/ml)	% Viable Hela cell	% Hela cell death	% Viable Lymphocytes	% Lymphocytes death
0.5	41.53 \pm 4.01	58.47	44.85 \pm 2.77	55.15
0.2	56.62 \pm 3.93	43.38	48.81 \pm 1.34	51.19
0.1	60.73 \pm 2.62	39.27	54.10 \pm 1.67	45.90
0.02	78.66 \pm 6.18	21.34	59.76 \pm 1.24	40.24
0.01	88.39 \pm 3.16	11.61	75 \pm 6.38	25.00
0	100 \pm 0.00	0	100 \pm 0.00	0
IC ₅₀	0.327 mg/ml		0.177 mg/ml	

Conclusion

Plants contain a wide variety of antioxidant phytochemicals or bioactive molecules, which can neutralize the free radicals and thus retard the progress of many chronic diseases. The intake of natural antioxidants has been associated with reduced risk of cancer, cardiovascular disease, diabetes and diseases associated with ageing. It can be concluded from the above results that *R. hemisphaerica* extract exhibited antimicrobial activity only against *S. aureus*. The extracts provided better antioxidative activity as compared with

synthetic antioxidants. The extracts were toxic to the Hela cells as well as human lymphocytes. Hence, the *R. hemisphaerica* extract may be exploited as a natural antioxidant and health promoting agent.

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