Antioxidative Properties and Toxicity of White Rose Extract

Ali Dehghan Kashani[1], Iraj Rasooli[2],Mohammad Bagher Rezaee[3], Parviz Owlia[4]*

Received:29.3.2011 *Accept:1.5.2011*

ABSTRACT

Background: Rosa damascena is a traditional medicinal plant used in Asia to treat several ailments. This study reports In vitro and In vivo antioxidative properties and toxicity of methanolic and aqueous extracts of white rose, an indigenous variety of Rosa damascena from Iran.

Methods: The antimicrobial activities, total phenolics, antioxidative properties and the blood sera Ferric-Reducing Antioxidant Power (FRAP) of the extracts were determined using standard methods. Acute and subchronic toxicity and cytotoxicity of the extracts were tested. Hematology and clinical chemistry parameters were investigated.

Results: S.aureus only was susceptible. The total phenol contents of the methanolic and aqueous extracts were 137.67±9.50 and 138.67±5.69 µg Gallic acid equivalent/mg sample respectively. DPPH scavenging and lipid peroxidation inhibition effects were higher than those of the synthetic antioxidants. A dose dependent FRAP was noted in blood sera of rats fed with the extracts. Cholesterol/HDL and LDL/HDL ratios, fasting glucose, blood urea nitrogen, creatinine and uric acid levels decreased. IC_{50} of 4.5 µg/ml was determined for cytotoxicity of the extract against Hela cell line.

Conclusion: Antioxidative activity and cytotoxicity of white rose suggests its promising applications as a natural antioxidant and health promoting agent for the treatment and prevention of free radicals associated diseases.

Key words: Antimicrobial, Antioxidant, Cytotoxicity, Rose, Rosa Damascena.

INTRODUCTION

Phytochemicals possess biological properties that promote human health and help reduce the risk of chronic disease (1). A wide diversity of phytochemicals exists within *Rosa* genera (2,3). It is well established that genotype or species may have a profound influence on the content of bioactive compounds in small fruits (4). One of the most important *Rosa* species is *Rosa damascena* Mill. which some of its varieties are very important for essential oil production and others are widely cultivated as garden roses (5). *R. damascena* is the most commonly used source of rose extracts and oil, but a number of other Rosa species (e.g., Rosa centifolia, Rosa gallica, Rosa alba, and Rosa rugosa) with similar chemical composition have been identified and used for therapeutic purposes (6). The physiological functions of *Rosaceae* may be partly attributed to their abundance of phenolics. Phenolic acids and flavonoids, known as bioactive agents, frequently occur in herbal plants (7). Phenolics possess a wide spectrum of biochemical activities, such as antioxidants, free-radical scavengers (8,9), anticancer (10), anti-inflammatory (11) and antimutagenic (12); however the antioxidative properties remain the core topic of investigation in recent years. Crude extracts of the plant parts rich in phenolics are increasingly of interest in the field of nutrition, health and medicine, because they retard oxidative degradation of lipids and thereby

1. *Microbiology student, Department of Biology, Shahed University, Tehran-Qom Express Way, Opposite Imam Khomeini's shrine, Tehran, Iran.*
2. *Medicinal Plants Research Center-Shahed University, Tehran-Qom Express Way, Tehran, Iran.*
3. *Research Institute of Forests and Rangelands, Tehran, Iran.*
4. *Faculty of Medicine, Shahed University, Tehran, Iran.*

** Corresponding author. E-mail address:rasooli@shahed.ac.ir*

improve the quality and nutritive value of foods (13). *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 (14). *Rosa* is a wide genus and each rose- growing country has its own endemic rose species. To the best of our knowledge, few data exist regarding properties of endemic Rose species in Iran. In this study we used white variety of rose which have not been studied in detail before.

MATERIALS AND METHODS

General

The major equipments used were, UV-1601PC spectrophotometer (Shimadzu, Japan) and ELISA reader DNM-9602G (Perlong group, Beijing, China). Microbial and cell culture media and laboratory reagents were from Merck, Germany. Other chemicals were of analytical grade and were from Sigma-Aldrich.

Preparation of extracts

The white rose samples, a variety of Rosa damascene, 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 12,000*g* 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 10^8 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 10^8 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). 50µl of the 20mg/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 2h. 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 (15). In brief, a 100 µl aliquot of dissolved extract was transferred to a volumetric flask, containing 46 ml distilled H_2O, to which was subsequently added 1 ml Folin–Ciocalteu reagent. After 3 mins, 3 ml of 2% Na_2CO_3 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:

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

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= $\ln(A_{initial}/ A_{sample})$/time (minutes)

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

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. (2009). 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 minutes 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 minutes. Absorbance for each tube was measured at 700 nm. The FRAP was expressed as gallic acid equivalents (GAE) in mg/g of samples used ($y = 16.66x+0.003$; $r^2 = 0.999$).

Serum Ferric reducing antioxidant power (FRAP)

The antioxidant power of blood serum was determined using FRAP assay (16). Briefly, 50 µl of the blood serum (normal as well as experimental cells) suspension was added to 1.5 ml of freshly prepared and pre-warmed (37 °C) FRAP reagent (300 mM acetate buffer, pH = 3.6, 10 mM TPTZ (tripyridyl-s-triazine) in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ in the ratio of 10:1:1) and incubated at 37 °C for 10 min. The absorbance of the sample was read against reagent blank (1.5 ml FRAP reagent + 50 µl distilled water) at 593 nm. Aqueous solutions of known Fe(II) concentration ($FeSO_4.7H_2O$) were used for calibration of the FRAP assay and antioxidant

power was expressed as µg/ml ($y = 0.002x$; $r^2 = 0.997$).

Acute and subchronic toxicity

In order to avoid any toxic effect of residual methanol in the extract and with respect to almost equal antioxidative properties of both extracts, this and cytotoxicity parts of the study were performed with the aqueous extract only. A 30-day oral toxicity study was conducted in Wistar rats (*Rattus norvegicus*; 180–200 g) to determine the potential of *the* extract to produce toxic effects. The rats of both sexes, were housed in temperature-controlled rooms and were given food and water *ad libitum* until used. The test extract was administered via oral gavage to the rats ($n = 10$ mice per group) orally at doses of 2.5, 5, 25 and 50 mg/kg/day corresponding approximately to doses of 0.5, 1, 5 and mg/animal/day respectively. The results obtained were compared with those for the control animals [0.9% saline]. The LD_{50} was calculated by the probit method by using SPSS 7.0 for Windows. To investigate the subchronic toxicity of the rose extract, after 30 days of oral administration to rats, the haematological and serum biochemistry parameters were evaluated. Blood samples were collected by puncture in the infraorbital plexus. The blood samples collected on day 0 and day 30 were used for determining red cell and leucocyte counts and for haemoglobin, haematocrit and biochemical parameter analysis. The serum concentrations of urea, creatinine, glutamic-oxalacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) and other parameters were determined by using commercial kits. The values obtained were compared within and between the groups.

Cytotoxicity assay

The human cervical carcinoma Hela cell line NCBI code No. 115 (ATCC number CCL-2) were procured from Pasteur Institute, Tehran-Iran. Human lymphocytes were obtained from healthy volunteers. The cytotoxicity assay was performed as stated elsewhere (17). 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 (18). Briefly, the cells (5×10^4) were seeded in each well containing 100µl 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 (19). 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.

Ethical Consideration

All the protocols that included animals were approved by the Ethics Committee in research of the Shahed University, Tehran-Iran. The animals were used as recommended by the guide for the care and use of laboratory animals from the National Academy Press (USA; 1996), which fulfils the principles for animal use in Iran.

Statistical analysis

All the experimental data are presented as mean ± SEM of three individual samples. 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. Data are presented as

percentage of free radical scavenging/inhibition lipid peroxidation on different concentration of the extract. IC_{50} (the concentration required to scavenge 50% of free radicals) value was calculated from the dose-response curves. All of the statistical analyses were performed with the level of significant difference between compared data sets being set at $p < 0.05$.

RESULTS

Antimicrobial activity

The antibacterial effects of the 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).

Table 1. Determination of growth inhibition zone of *S. aureus* exposed to white *Rose* extracts

	Mean Inhibition Zone (mm) 50µl (1 mg)/Well	Mean Inhibition Zone (mm) 50µl (0.5 mg)/Well
Methanolic extract	15±0.0	10.50±0.71
Aqueous extract	15±0.0	12.50±0.71

Total phenolics content(TPC)

The total phenol contents (TPC) of the methanolic and aqueous extracts were determined to be 137.67±9.50 and 138.67±5.69 µg Gallic acid equivalent /mg sample (GAE/mg) respectively (Table 2).

Table 2. Total phenolics of white *rose* 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)	97.2±0.11	43.8	137.67±9.50
Aqueous extract (1 mg/ml)	97.08±0.23	3.3	138.67±5.69
BHT 1mM	35.9±0.47	—	—
BHA 1mM	47.7±0.47	—	—
Trolox 1mM	34.5±0.4	—	—

Antioxidant activity

The antioxidant capacities of the rose extracts as assessed by different assay methods are summarized in Tables 2-4. The extracts exhibited a dose-dependent scavenging of DPPH radicals and 43.8 µg/ml and 3.3 µg/ml of the methanolic and aqueous extracts were sufficient to scavenge 50% of DPPH radicals respectively (Table 2). In the peresnt study DPPH scavenging effect (%) of the extracts were significantly higher than those of the synthetic antioxidants (Table 2). Lipid peroxidation inhibition activity (LPI) of *the* 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 FRAP of the aqueous extracts tested in blood sera of the rats gavaged with a daily dose of 50, 25, 5 and 2.5mg/kg showed a dose dependent and increased levels of ferric-reducing antioxidant power as compared with the control group (Table 5).

Table 3. Lipid peroxidation inhibition activity of white rose extracts determined by β-carotene bleaching assay at different time intervals

Antioxidant agents	30 minutes	60 minutes	90 minutes
White Rose (methanolic extract)	53.82±2.76	80.04±2.92	83.17±2.58
White Rose (aqueous extract)	51.71±3.53	79.41±3.19	82.23±3.06
BHT 1mM	54.84±2.55	78.54±2.46	81.15±2.35
BHA 1mM	54.93±2.65	78.49±2.43	81.48±2.50

Table 4. Ferric-Reducing Antioxidant Power (FRAP) of white rose extracts (Gallic acid equivalent (mg/g))

	Methanolic extract	Aqueous extract
1mg/ml	97.09±1.14	98.07±0.56
0.5mg/ml	48.25±0.76	49.95±0.93
0.25mg/ml	23.82±0.27	24.58±0.64
0.1mg/ml	9.54±0.41	9.46±0.33
Mean GAE (mg/g)	96.134±4.25	98.63±2.51

Table 5. Serum Ferric-Reducing Antioxidant Power (FRAP) assay of white rose extracts

	$FeSO_4.7H_2O$ equivalent (μg/ml)	Test/Control Ratio (%)
10mg/ml	313.01±9.44	153.89
5mg/ml	232.56±5.51	114.34
1mg/ml	203.40±8.93	111.79
0.5mg/ml	213.72±5.91	105.07
(Control)	227.37±4.95	100.00

Acute and subchronic toxicity

There was increased body weight gain in test groups as compared with the control. However the percent weight gains were not statistically significant. Significant decrease in total white blood cell (WBC) was noted at highest and lowest concentrations of the extracts while platelet counts were significantly increased in all test groups. Fasting glucose, SGOT and SGPT levels were significantly decreased and alkaline phosphatase levels were significantly increased in all test groups (Table 6). Clinical chemistry parameters also showed increased levels of triglycerides. This increase was statistically significant only in high dose group. Interestingly, cholesterol/HDL ratio and LDL/HDL ratio were also higher in the sera of the high dose group while these levels were significantly decreased in other three doses of 25, 5 and 2.5 mg/kg/day groups.

Table 6. Mean hematology and clinical chemistry values of rats blood samples gavaged with white *rose* aqueous extract

Parameters	Control	50mg/Kg/Day %Change P value	25mg/Kg/Day %Change P value	5mg/Kg/Day %Change P value	2.5mg/Kg/Day %Change P value
Initial Body weight (g)	142.50±2.9	188±25.17 132.16 0.013	190±16.33 133.33 0.001	188±25.66 132.16 0.014	181±25.29 127.19 0.023
Final Body weight (g)	157.50±5	216±43.68 137 0.039	227±27.54 144 0.002	210±30 133 0.016	210±36.51 133 0.029
Weight gain (%)	110.53±2	114±7.73 3.86 0.391	119.57±6.65 9.05 0.0554	111.49±3.41 0.96 0.703	115.50±6.18 4.97 0.196
Erythrocyte count (RBC) ($\times 10^6$/lL)	7.23±1.25	7.72±0.92 106.81 0.592	9.08±0.29 125.64 0.027	7.84±0.38 108.51 0.455	8.21±0.76 113.67 0.224
Total white blood cell (WBC) and differential leukocyte count ($\times 10^3$/µL)	9400±668	6533±929 69.50 0.004	11700±372 124.47 0.27	6933.33±212 73.76 0.075	7400±114 78.72 0.023
Hemoglobin concentration (HGB) (g/dL)	12.78±1.4	13.53±1.6 105.94 0.540	15.25±0.55 119.37 0.019	13.93±0.96 109.07 0.288	14.25±1.17 111.55 0.164
Hematocrit (HCT) (%)	39.63±1.7	39.30±3.41 99.18 0.873	43.55±1.35 109.91 0.012	38.90±3.41 98.17 0.724	39.98±3.80 100.88 0.872
Platelet count (PLT) ($\times 10^3$/µL)	238500 ±1658	481333.33±4747 201.82 0.0002	446500±46200 187.21 0.0001	513333±7427 215.23 0.0007	781500±1244 327.67 0.001
Red Cell Distribution Width [RDW (%)]	14.75±2.14	15.83±2.66 107.34 0.574	14.95±0.06 101.36 0.857	14.73±0.12 99.89 0.99	16.05±1.22 108.81 0.331
Mean Platelet Volume (MPV)	7.600.56	8.23±0.99 108.33 0.324	7.35±0.34 96.71 0.474	7.77±0.86 102.19 0.767	7.43±0.38 97.70 0.623
Mean corpuscular volume (MCV) (fL)	52.93±3.84	50.53±2.06 95.48 0.38	47.93±0.38 90.55 0.041	49.60±2.67 93.72 0.26	48.68±1.94 91.97 0.096
Mean corpuscular hemoglobin (MCH) (pg)	17.78±1.37	17.53±0.87 98.64 0.802	16.75±0.17 94.23 0.189	17.40±0.80 97.89 0.7	17.38±0.62 97.75 0.615

Mean corpuscular hemoglobin Concentration [MCHC (g/dL)]	33.75±1.95	35.40±0.62 104.89 0.224	34.98±0.22 103.63 0.2581	35.90±1.30 106.37 0.162	35.78±0.88 106 0.107
Fasting glucose (GLUC) (mg/dL)	221±7.79	196±5 88.69 0.005	201±15.38 90.95 0.059	202.33±11.59 91.55 0.05	190.50±20 86.20 0.030
Blood Urea nitrogen (BUN) (mg/dL)	60±5.96	61.67±2.52 102.78 0.673	70.53±7.85 117.54 0.076	70.50±3.58 117.50 0.044	54±7.80 90 0.267
Blood creatinine (CREA) (mg/dL)	0.64±0.1	0.49±0.03 76.64 0.063	0.46±0.06 72.05 0.024	0.42±0.02 65.62 0.016	0.41±0.09 63.78 0.014
Uric acid	8.18±2.45	7.20±0.46 88.07 0.535	2.08±0.43 25.38 0.002	1.83±0.15 22.43 0.007	1.90±0.76 23.24 0.003
Total cholesterol(CHOL) (mg/dL)	75.75±1	76.67±4.73 101.21 0.712	75±15.53 99.01 0.926	75.67±6.03 99.89 0.98	75.75±3.30 100 1
Triglycerides (TRIG) (mg/dL)	45±9.2	82.67±3.79 183.70 0.001	60±10.23 133.33 0.072	51.67±5.03 114.81 0.314	53.25±9.64 118.33 0.262
HDL	45.50±4	43.30±2.54 95.16 0.447	62.03±7.46 136.32 0.008	53.03±7.89 116.56 0.154	57.40±5.63 126.15 0.014
LDL	15.05±1.9	19.97±1.61 132.67 0.016	7.60±2.17 50.50 0.002	12.63±1.76 83.94 0.15	7.15±2.03 47.51 0.001
Cholesterol/HDL ratio	1.68±0.16	1.78±0.2 106.10 0.485	1.20±0.15 71.85 0.005	1.44±0.11 85.79 0.084	1.33±0.13 79.26 0.015
LDL/HDL ratio	0.33±0.03	0.46±0.06 140.09 0.007	0.12±0.03 37.10 0.0001	0.24±0.07 73.83 0.058	0.13±0.04 37.86 0.0001
SGOT	530.75±68	404±47.51 76.12 0.042	253.28±53.85 47.72 0.0007	251.33±30.89 47.35 0.001	220.75±25.2 41.59 0.0001
SGPT	236.75±9	140±20 59.13 0.026	93.50±12.26 39.49 0.001	111±7.81 46.88 0.008	78.50±7.33 33.16 0.001
Alkaline phosphatase (ALKP) (U/L)	136.75±3	309.67±28.29 226.45 0.0008	241.25±39.23 176.42 0.006	305.33±133.64 223.28 0.055	222.83±36.27 162.94 0.013

Cytotoxicity

The extract displayed an excellent cytotoxic action towards the human tumor cell line (Table 7). The aqueous extract at 0.5 mg/ml concentration destructed Hela cells and lymphocytes by 94.72% and 47.08% with IC_{50} of 0.0045 and 115.7mg/ml respectively. (Table 7).

Table 7. Cytotoxicity assay of white Rose aqueous extract

Extract Dilutions (mg/ml)	% Viable Hela cell	% Hela cell death	Extract Dilutions (mg/ml)	% Viable Lymphocytes	% Lymphocytes death
0.5	5.28±0.82	94.72	100	52.92±1.13	47.08
0.2	14.12±1.26	85.88	40	60.87±3.11	39.13
0.1	28.15±2.47	71.85	20	66.04±6.09	33.96
0.02	32.99±1.92	64.6	4	68.70±6.81	31.3
0.01	41.90±1.61	58.1	2	76.46±9.94	23.54
0.005	48.2±1.58	51.8	0	100±0.0	0
0.0025	53.8±0.78	46.2	—	—	—
0	100±0.00	0	—	—	—
IC_{50}	0.0045 mg/ml			115.7 mg/ml	

DISCOSSION

The results showed that only S. aureus was susceptible to antibacterial effect of the extract. Susceptibility of *S.aureus* is consistent with those reported earlier (20,21). The resistance of *E.coli* in the present study confirms report of other investigators (22). It is suggested that the phenolics compounds which are antioxidants are responsible for the antibacterial activity (23). TPC of methanolic extracts of *R. damascena* flowers was reported in other study to contain 145 ± 1.4 mg GAE/g (24). This is almost similar to our results. The higher phenolic acid levels in methanolic extracts could be due to extraction of both nonpolar and semipolar soluble phenolic acids. 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 (25). The methods involving an electron-transfer reaction include the total phenolics assay using Folin–Ciocalteu reagent, the TEAC and the DPPH radical-scavenging assay. Fresh and spent *Rosa hemisphaerica* flower extracts showed 74.51±1.65 and 75.94±1.72% antiradical activities at 100ppm. (22) which are lower than those of our extracts. The DPPH radical scavenging is a sensitive antioxidant assay and is independent of substrate polarity (26). 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 (27). 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 (24). 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 (28). The high antioxidant activity of the extracts could be attributed to its high phenolic content. This preliminary study indicates the interesting antioxidative stress activity of white rose suggesting its promising applications as a medicinal source for the treatment and prevention of free radicals associated diseases. There were considerable treatment-related effects in hematology and clinical chemistry

parameters (Table 6). Decrease in some blood parameters such as fasting glucose, blood urea nitrogen (BUN), creatinine (CREA) and uric acid is suggestive of promising therapeutic potentials of the extract at lower doses. Thus, white rose extract with a high phenolic content and good antioxidant activity can be supplemented for nutritional purposes. Oral administration of acetone fraction at 50mg/kg body weight significantly reduced the serum alkaline phosphatase (ALP), glutamine pyruvate transaminase (GPT) and glutamine oxaloacetate transaminase (GOT) activity and lipid peroxide level in rats receiving an acute dose. This indicated that *Rosa damascena* could protect against induced hepatotoxicity, possibly by its free radical scavenging activity (29). The cytotoxicity results are interesting on the part of efficacy of lower concentration of the extract against cancer cells rather than the higher concentration required to kill healthy cells. Although all in vitro experiments hold limitations with regards to possible in vivo efficacy, the results of this study are very promising with regards to possible anti-neoplastic chemotherapy and form a very sound basis for future research. Some reports support the relationship of cytotoxicity with antioxidant activity (30). So the antioxidant activity of rose extract might contribute to its cytotoxic activity.

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 associated with oxidative stress and reactive oxygen species (ROS). 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 white variety of *R. damascena* extract exhibited antimicrobial activity only against *S.aureus*. The extract provided better antioxidative activity as compared with synthetic antioxidants, which provides a way of screening antioxidants for foods, cosmetics and medicine. Hence, the *R. damascena* extract may be exploited as a natural antioxidant and health promoting agent that can conveniently find its appropriate therapeutic applications.

ACKNOWLEDGMENTS

The authors wish to thank Mr. Mohammad Ali Rezaee, the managing director of Iman Mehr company (Tehran-Iran) and Iranian Ministry of Health for the sanction of research grants to conduct the present study.

REFERENCES

1. Curin Y, Andriantsitohaina R. Polyphenols as potential therapeutical agents against cardiovascular diseases. Pharmacological Reports 2005;57(SUPPL.):97-107.
2. Olsson ME, Andersson S, Werlemark G, Uggla M, Gustavsson KE. Carotenoids and phenolics in rose hips. Acta Horticulturae 2005;490:249-53.
3. Uggla M, Gustavsson KE, Olsson ME, Nybom H. Changes in colour and sugar content in rose hips (Rosa dumalis L. and R. rubiginosa L.) during ripening. J Hortic Sci Biotechnol 2005;80(2):204-8.
4. Anttonen MJ, Karjalainen RO. Environmental and genetic variation of phenolic compounds in red raspberry. J Food Compos Anal 2005;18(8):759-69.
5. Rusanov K, Kovacheva N, Vosman B, Zhang L, Rajapakse S, Atanassov A, Atanassov I. Microsatellite analysis of Rosa damascena Mill. accessions reveals genetic similarity between genotypes used for rose oil production and old Damask rose varieties. Theor Appl Genet 2005;111(4):804-9.
6. Rangaha MK. Rose - Rosa damascena 'Trigintipetala. Crop Food Res Broad Sheet 2001;29.
7. Wen D, Li C, Di H, Liao Y, Liu H. A universal HPLC method for the determination of phenolic acids in compound herbal medicines. J Agric Food Chem 2005;53(17):6624-9.
8. Leenen R, Roodenburg AJC, Tijburg LBM, Wiseman SA. A single dose of tea with or without milk increases plasma antioxidant activity in humans. European Journal of Clinical Nutrition 2000;54(1):87-92.
9. Ng TB, Liu F, Wang ZT. Antioxidative activity of natural products from plants. Life Sciences 2000;66(8):709-23.

10. Ren W, Qiao Z, Wang H, Zhu L, Zhang L. Flavonoids: Promising anticancer agents. Medicinal Research Reviews 2003;23(4):519-34.
11. Crespo ME, lvez J, Cruz T, Ocete MA, Zarzuelo A. Anti-inflammatory activity of diosmin and hesperidin in rat colitis induced by TNBS. Planta Medica 1999;65(7):651-3.
12. Miyazawa M, Okuno Y, Nakamura SI, Kosaka H. Antimutagenic activity of flavonoids from Pogostemon cablin. J Agric Food Chem 2000;48(3):642-7.
13. Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M. Antioxidant activity of plant extracts containing phenolic compounds. J Agric Food Chem 1999;47(10):3954-62.
14. Jafari M, Zarban A, Pham S, Wang T. Rosa damascena decreased mortality in adult Drosophila. J Med Food 2008;11(1):9-13.
15. Singleton VL, Orthofer R, Lamuela-Raventos RM: Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. SAN DIEGO, ACADEMIC PRESS INC, 1999.
16. Benzie IFF, Strain JJ: [2] Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration; in Lester P, (ed): Methods in Enzymology
Oxidants and Antioxidants Part A. San Diego, Academic Press, 1999, pp 15-27.
17. Plumb JA, Milroy R, Kaye SB. Effects of the pH dependence of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide-formazan absorption on chemosensitivity determined by a novel tetrazolium-based assay. Cancer Res 1989;49(16):4435-40.
18. Lau CBS, Ho CY, Kim CF, Leung KN, Fung KP, Tse TF, Chan HHL, Chow MSS. Cytotoxic activities of Coriolus versicolor (Yunzhi) extract on human leukemia and lymphoma cells by induction of apoptosis. Life Sci 2004;75(7):797-808.
19. Sylvestre M, Legault J, Dufour D, Pichette A. Chemical composition and anticancer activity of leaf essential oil of Myrica gale L. Phytomedicine 2005;12(4):299-304.
20. Aktan S, Sagdic O. Dried rose (Rosa damascena Mill.) dreg: An alternative litter material in broiler production. S Afr J Anim Sci 2004;34(2):75-9.
21. Aridogan BC, Baydar H, Kaya S, Demirci M, Ozbasar D, Mumcu E. Antimicrobial activity and chemical composition of some essential oils. Arch Pharmacal Res 2002;25(6):860-4.
22. Ozkan G, Sagdic O, Baydar NG, Baydar H. Note: Antioxidant and antibacterial activities of Rosa damascena flower extracts. Food Sci Technol Int 2004;10(4):277-81.
23. Cushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents 2005;26(5):343-56.
24. Kumar N, Bhandari P, Singh B, Bari SS. Antioxidant activity and ultra-performance LC-electrospray ionization-quadrupole time-of-flight mass spectrometry for phenolics-based fingerprinting of Rose species: Rosa damascena, Rosa bourboniana and Rosa brunonii. Food and Chemical Toxicology 2009;47(2):361-7.
25. Huang D, Boxin OU, Prior RL. The chemistry behind antioxidant capacity assays. J Agric Food Chem 2005;53(6):1841-56.
26. Yamaguchi T, Takamura H, Matoba T, Terao J. HPLC Method for Evaluation of the Free Radical-scavenging Activity of Foods by Using 1,1-Diphenyl-2-picrylhydrazyl. Bioscience, Biotechnology, and Biochemistry 1998;62(6):1201-4.
27. Ho SC, Tsai TH, Tsai PJ, Lin CC. Protective capacities of certain spices against peroxynitrite-mediated biomolecular damage. Food and Chemical Toxicology 2008;46(3):920-8.
28. Lim TY, Lim YY, Yule CM. Evaluation of antioxidant, antibacterial and anti-tyrosinase activities of four Macaranga species. Food Chem 2009;114(2):594-9.
29. Achuthan CR, Babu BH, Padikkala J. Antioxidant and hepatoprotective effects of Rosa damascena. Pharm Biol 2003;41(5):357-61.
30. Hou J, Sun T, Hu J, Chen S, Cai X, Zou G. Chemical composition, cytotoxic and antioxidant activity of the leaf essential oil of Photinia serrulata. Food Chem 2007;103(2):355-8.