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Original Article

Cytobiochemical Potentials of *Rosa damascena* Mill. Extract

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ABSTRACT

Background and Objectives: **There is a profound inclination among people toward consumption of herbs and herbal products. Some of these products are harmful while health-promoting potentials of some others should be discovered. In the present study the antibacterial, antioxidant, acute and subchronic and cancer cell toxicity of methanolic and aqueous extracts of *Rosa damascena* Mill. was studied.**

Materials and Methods: **Antimicrobial activities were determined by agar disc diffusion method. Total phenol content was 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 or known Fe (II) concentration for rose extracts and blood sera respectively. Acute and subchronic toxicity and cytotoxicity of the extracts were tested using animal model or Hela cells. Hematology and clinical chemistry parameters were noted.**

Results: ***Staphylococcus aureus*** **was found susceptible. The total phenol contents of the methanolic and aqueous extracts were 132.67±3.51 and 117.33±6.81 µg Gallic acid equivalent/mg sample, respectively. Antioxidative effects were higher than those of the synthetic antioxidants were. A dose dependent levels of FRAP was noted in blood sera of rats gavaged with the extracts. Decrease in cholesterol/HDL and LDL/HDL ratios, fasting glucose, blood urea nitrogen, creatinine and uric acid is suggestive of promising therapeutic potentials of the extract. Inhibitory concentration of 50% (IC_{50}) of 4.5 µg/ml was determined for cytotoxicity of the extract against Hela cell line.**

Conclusion: The results suggest application of rose extract as a natural antioxidant and health-promoting agent.

Key words: Rose, Antimicrobial Agents, Antioxidant; Cytotoxicity

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Introduction

Various phytochemicals from herbs and herbal extracts with human health promoting or biological properties are available that reduce the risk of chronic disease (1). Rose has been registered with more than 150 species and 2000 cultivars (2). Members of the Rosaceae family possess food and medicinal applications since antiquity. Except for *E. coli* O157:H7, the fresh (FF) and spent (SF) *Rosa damascena* flower extracts were reported to be effective bactericidal against all the bacteria with the strongest effects against *S. enteritidis* and *M. smegmatis*, respectively (3). Antimicrobial activity of *Rosa damascena* was recorded only to *Staphylococcus aureus* (4). Abundance of phenolics and flavonoids is the major reason for bioactivity and physiological functions of *Rosaceae* as well as herbal plants (5). The correlation coefficients between antioxidant activity, on the one hand, and the contents of total phenols and of gallic acid in various rose cultivars, on the other hand, were 0.79 and 0.81, respectively. A separate or combined form of dried rose petals may be used for preparing antioxidant-rich caffeine-free beverages (6) .Phenolics possess a wide spectrum of biochemical activities, such as antioxidants, free-radical scavengers (7), anticancer (8) and anti-inflammatory (9); however, the antioxidative properties remain the core topic of investigation in recent years.

Phenolic rich crude plant parts are of profound interest in nutrition, health and medicine fields for their retardive activity of oxidative degradation of lipids and thereby improvemet of the quality and nutritive value of foods (10). There was a significant decrease in mortality rate in male and female *Drosophila* supplemented with *Rosa damascena* extract. The anti aging effects were not associated with metabolic rate or other common confounds of anti aging potentials (11) .Phenolic constituents of wild growing *Rosa damascena* were not characterized prior to the recent report on determination of polyphenols in *R. damascena*, *R. bourboniana* and *R. brunonii employing an* -HPLC method (12).

The aim of this study was to determine the health promoting potentials of *Rosa damascena* Mill. extracts grown extensively and consumed widely in Iran, Iran. Thus, herein we report the assessment of antimicrobial, antioxidant capacities, total phenolics, acute and subchronic toxicity, and human cell anti tumor cytotoxicity of *Rosa damascena* Mill. 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 damascena collected from the natural rose gardens of Kashan City, Central 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. Five hundred 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

The micro orgamismas used were *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), *Streptococcus faecalis* (PTCC 33186), *Pseudomonas aeruginosa* (ATCC 8830) and *Klebsiella pneumoniae* (ATCC 13883). Bacterial suspensions were made in Brain Heart Infusion (BHI) broth to a concentration of approximately 10^8 cfu/ml. The suspension concentrations were measured spectrophotometrically. 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 filtering sterilized, as and when needed, using 0.45µ sterile filter (13).

Disc diffusion method

The agar disc diffusion method (13) was employed for the determination of antimicrobial activities of the extracts in question. Briefly, 0.1 ml from 10^8 CFU/mL bacterial suspensions was spread on the Mueller Hinton Agar (MHA) plates. The agar was bored with a sterile borer (6 mm in diameter). Fifty µl of the 20mg/ml and 10 mg/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 (14). In brief, a 100 µl aliquot of dissolved extract was transferred to a volumetric flask, containing 46.0 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 (13):

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

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 (13):

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 (15). 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 ($y = 16.667x+0.0038$; $r^2 = 0.9991$).

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 ^oC) FRAP reagent (300 mM acetate buffer, pH = 3.6, 10 mM TPTZ (tripyridyl-s-triazine) in 40 mM HCl and 20 mM FeCl3.6H2O in the ratio of 10:1:1) and incubated at 37 ^oC 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 ($y = 0.0025x+0.0005$; $r^2 = 0.9976$).

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 aqueous extract of *R. damascene* only. A 30-day oral toxicity study was conducted in Wistar rats (*Rattus norvegicus*; 180–200 g) to determine the potential of *R. damascena* methanolic 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 (17).

Cytotoxicity assay

The 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 (17). 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 (18). 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 ± SEM of three individual samples. Data are presented as percentage of free radical scavenging/inhibition lipid peroxidation on different concentration of *Rosa damascena* extract. IC_{50} (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

Antimicrobial activity

The antibacterial effect of *Rosa damascena* 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 *Rosa damascena* extracts, the total phenolics of the extracts and mean inhibition of DPPH free radical (%)

Extracts and synthetic antioxidants	Mean Inhibition Zone (mm) 50µl (1 mg)/Well	Mean Inhibition Zone (mm) 50µl (0.5 mg)/ Well	DPPH scavenging effect (%)	DPPH (IC_{50}) (µg/ml)	Total phenolic content GAE µg Gallic acid/ mg sample
Methanolic extract	14.67±0.58	11.00±0.00	97.25±0.07	49	132.67±3.51
Aqueous extract	14.67±0.58	11.50±0.71	97.75±0.16	40	117.33±6.81
BHT 1mM	—	—	35.9±0.47	—	—
BHA 1mM	—	—	47.7±0.47	—	—
Trolox 1mM	—	—	34.5±0.4	—	—

Total Phenolics Content(TPC)

The total phenol contents (TPC) of the methanolic and aqueous extracts of *R. damascena* flower were determined to be 132.67±3.51 and 117.33±6.81 µg Gallic acid equivalent /mg sample (GAE/mg) respectively (Table 1).

Antioxidant activity

The antioxidant capacities of the rose extracts as assessed by different assay methods are summarized in Tables 1-3. *Rosa damascena* extracts exhibited a dose-dependent scavenging of DPPH radicals and 49 µg/ml and 40 µg/ml of the methanolic and aqueous extracts were sufficient to scavenge 50% of DPPH radicals respectively (Table 1). In the peresnt study DPPH scavenging effect (%) of the extracts were significantly higher than those of the synthetic antioxidants (Table 1). Lipid peroxidation inhibition activity of *Rosa damascena* extracts determined by β-carotene bleaching assay revealed statistically equal potency to the standard BHT and BHA (Table 2). Ferric-reducing antioxidant power (FRAP) of the methanolic and aqueous extracts were determined as 103.9±7.86 mg/g and 97.6±3.3 mg/g respectively. 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 to the control group (Table 3).

Table 2. Lipid peroxidation inhibition activity of *Rosa damascena* extracts determined by β-carotene bleaching assay at different time intervals

Antioxidant agents	30 minutes	60 minutes	90 minutes
***Rosa damascena* methanolic extract**	53.59±2.87	79.77±2.59	82.85±2.53
***Rosa damascena* aqueous extract**	48.71±2.07	77.54±2.38	80.61±2.40
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 3. Serum Ferric-Reducing Antioxidant Power (FRAP) assay of *Rosa damascena* extracts

	FeSO4.7H2O equivalent (µg/ml)	Test/Control Ratio (%)
10mg/ml	349.81±6.39	175.18
5mg/ml	237.12±4.82	118.75
1mg/ml	217.69±6.84	109.02
0.5mg/ml	211.99±9.25	106.16
(Control)	199.68±4.65	100.00

Acute and subchronic toxicity

There were considerable treatment-related effects in hematology and clinical chemistry parameters (Table 4). There was increased body weight in test groups as compared to 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 4). 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. This, and decrease in other parameters such as fasting glucose, blood urea nitrogen (BUN), creatinine (CREA) and uric acid (Table 4) is suggestive of promising therapeutic potentials of *R.damascena* extract at lower doses.

Table 4. Mean hematology and clinical chemistry values of rats blood samples fed with *Rosa damascena* aqueous extract

Parameters		**50mg/Kg/Day**	**25mg/Kg/Day**	**5mg/Kg/Day**	**2.5mg/Kg/Day**
		%Change	**%Change**	**%Change**	**%Change**
	Control	***P* value**	***P* value**	***P* value**	***P* value**
Initial Body weight (g)		188.33±25.17	190±16.33	188.33±25.66	181.25±25.29
		132.16	133.33	132.16	127.19
	142.50±2.9	0.013	0.001	0.014	0.023
Final Body weight (g)		216.67±43.68	227.50±27.54	210±30	210±36.51
		137.57	144.44	133.33	133.33
	157.50±5	0.039	0.002	0.016	0.029
Weight gain (%)		114.39±7.73	119.57±6.65	111.49±3.41	115.50±6.18
		3.86	9.05	0.96	4.97
	110.53±2	0.391	0.0554	0.703	0.196

Erythrocyte count (RBC) ($\times 10^6$/lL)		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
	7.23±1.25				
Total white blood cell (WBC) and differential leukocyte count ($\times 10^3$/µL)		6533.33±929 69.50 0.004	11700±372 124.47 0.27	6933.33±212 73.76 0.075	7400±114 78.72 0.023
	9400±668				
Hemoglobin concentration (HGB) (g/dL)		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
	12.78±1.4				
Hematocrit (HCT) (%)		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
	39.63±1.7				
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 (%)]		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
	14.75±2.14				
Mean Platelet Volume (MPV)		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
	7.600.56				
Mean corpuscular volume (MCV) (fL)		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
	52.93±3.84				
Mean corpuscular hemoglobin (MCH) (pg)		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
	17.78±1.37				
Mean corpuscular hemoglobin Concentration [MCHC (g/dL)]		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
	33.75±1.95				
Fasting glucose (GLUC) (mg/dL)		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
	221±7.79				
Blood Urea Nitrogen (BUN) (mg/dL)		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
	60±5.96				
Blood creatinine (CREA) (mg/dL)		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
	0.64±0.1				
Uric acid (mg/dL)		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
	8.18±2.45				
Total cholesterol(CHOL) (mg/dL)		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
	75.75±1				
Triglycerides (TRIG) (mg/dL)		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
	45±9.2				

HDL		43.30±2.54	62.03±7.46	53.03±7.89	57.40±5.63
(mg/dL)		95.16	136.32	116.56	126.15
	45.50±4	0.447	0.008	0.154	0.014
LDL		19.97±1.61	7.60±2.17	12.63±1.76	7.15±2.03
(mg/dL)		132.67	50.50	83.94	47.51
	15.05±1.9	0.016	0.002	0.15	0.001
Cholesterol/HDL ratio		1.78±0.2	1.20±0.15	1.44±0.11	1.33±0.13
		106.10	71.85	85.79	79.26
	1.68±0.16	0.485	0.005	0.084	0.015
LDL/HDL ratio		0.46±0.06	0.12±0.03	0.24±0.07	0.13±0.04
		140.09	37.10	73.83	37.86
	0.33±0.03	0.007	0.0001	0.058	0.0001
SGOT		404±47.51	253.28±53.85	251.33±30.89	220.75±25.2
(IU/L)		76.12	47.72	47.35	41.59
	530.75±68	0.042	0.0007	0.001	0.0001
SGPT		140±20	93.50±12.26	111±7.81	78.50±7.33
(mg/dL) (IU/L)		59.13	39.49	46.88	33.16
	236.75±9	0.026	0.001	0.008	0.001
Alkaline phosphatase		309.67±28.29	241.25±39.23	305.33±133.64	222.83±36.27
(ALKP) (U/L)		226.45	176.42	223.28	162.94
	136.75±3	0.0008	0.006	0.055	0.013

Cytotoxicity

The aqueous extract of *R.damascena* at 0.5 mg/ml concentration destructed cells by 74.11% (Table 5). The 50% cytotoxic concentration was found to be 4.5 µg/ml.

Table 5. Cytotoxicity assay of *Rosa damascena* aqueous extract

Extract Dilutions (mg/ml)	٪ Viable Hela cell line	٪ Death
0.5	25.89 ± 0.36	74.11
0.2	26.67 ± 0.48	73.33
0.1	28.20 ± 1.08	71.80
0.02	30.24 ± 1.02	69.76
0.01	38.74 ± 1.22	61.26
0.005	47.5 ± 0.78	52.5
0.0025	55.4 ± 0.92	44.6

Discussion

Susceptibility of *S. aureus* is consistent with those reported earlier (4). Both water and ethanolic extracts of *R. damascena* were effective on methicillin-resistant (MRSA). MIC and MBC values of 0.395 to 0.780 mg/ml and 1.563 to 3.125 mg/ml, respectively, were reported for the greatest antimicrobial activity of ethanolic extract of *R. damascena* (19) .The resistance of *E. coli* in the present study confirms report of other investigators (3). The anti bacterial activity has been attributed to the antioxidants with phenolic content (20). The total phenol contents (TPC) of the methanolic and aqueous extracts of *R. damascena* flower (Table 1) were comparable to other study (21). The total phenolic contents of fresh (FF) and spent (SF) *Rosa damascena* flower extracts were reported as 276.02 ± 2.93 and 248.97 ± 2.96 mg GAE/g respectively (3) which is almost double amount of our findings. Presence of nonpolar and semipolar soluble phenolic acids could be a reason

for higher phenolic acid levels in methanolic extracts. Various methods, based on the nature of the reaction, are available for evaluating the antioxidant capacity of biological samples (22). The methods involving an electron-transfer reaction include the total phenolics assay using Folin–Ciocalteu reagent, the TEAC and the DPPH radical-scavenging assay. The IC_{50} value for the methanolic extract of *R. damascena* was reported relatively as low as 21.4 µg/ml (21). IC_{50} of 13.75, 135 and 410µg/ml were reported for superoxide radical production, hydroxyl radical generation and lipid peroxide formation respectively for partially purified acetone fraction of *R. damascena* Mill. flower (23). Fresh and spent *Rosa damascena* flower extracts showed 74.51±1.65 and 75.94±1.72% antiradical activities at 100ppm. (3) which are lower than those of our extracts. DPPH is a stable free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. For each spice, there was a significant correlation between phenolic content and DPPH scavenging capacity (24). 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 (21). Lipid peroxide inhibition measures hydrophobic character of antioxidant molecules while both types of antioxidants, hydrophobic and hydrophilic, are determined by measurement of total phenolics content (15). The high phenolic content of *R. damascena* extracts could be responsible for high antioxidant activity. In vivo evaluation of antioxidant effects of ethanol extract of *R. damascena* petals performed by oral administration at doses of 50, 75, 100 and 200 mg/kg/day in rats for 10 days showed the highest activity with the dose of 200 mg/kg/day (25) .This preliminary study indicates the interesting anti oxidative stress activity of *R. damascene* suggesting its positive applications as a medicinal source for the treatment and prevention of free radicals associated diseases. *R. damascena* extract with a high phenolic content and good antioxidant activity can be supplemented for nutritional purposes. A significant reduction was noted in glutamine pyruvate transaminase, glutamine oxaloacetate transaminase, lipid peroxide and alkaline phosphatase in sera of rats administered orally with 50mg/kg body weight of acetone fraction of *R. damascene. This suggests protective activity* by free radical scavenging property *of the extract against* hepatotoxicity (23). The extract displayed an excellent cytotoxic action towards the human tumor cell line. In spite of the limitations of all in vitro studies with respect to in vivo impact, the present results are very promising as far as anti-neoplastic chemotherapy is concerned. This further forms a firm base for future research. Some reports support the relationship of cytotoxicity with antioxidant activity (26). Therefore, the antioxidant activity of *R. damascena* extract might contribute to its cytotoxic activity.

Conclusion

Phytochemicals are capable of neutralizing the free radicals and preventing a number of chronic diseases related to reactive oxygen species and oxidative stress. 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. damascena* extract exhibited antimicrobial activity only against *S.aureus*. The extracts 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 finds its appropriate therapeutic applications.

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