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### EVALUATION OF ANTIULCER AND *IN-VITRO* ANTIOXIDANT ACTIVITIES OF *ROSA DAMASCENA MILL*

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#### ABSTRACT

The present study was carried out on the plant of *Rosa damascena* Mill. having several pharmacological actions including anti-HIV, antibacterial, antioxidant, antitussive, hypnotic, anti-diabetic and antiulcer activity. In the present study anti-ulcer and *in-vitro* antioxidant activities of methanol, acetone and ethyl acetate extracts of flowers of *Rosa damascena* Mill. was evaluated. Anti-ulcer activity was evaluated by pylorus ligation, ethanol induced methods and *in-vitro* antioxidant activity evaluated by DPPH, Hydroxyl, Nitric oxide, H<sub>2</sub>O<sub>2</sub> methods respectively. Methanol extract showed significant anti-ulcer activity compared with standard drug Ranitidine HCl. In antioxidant studies methanol extract shows the significant activity in DPPH method and hydroxyl radical scavenging activity, acetone extract shows significant activity in nitric oxide method and ethyl acetate extract had better activity in H<sub>2</sub>O<sub>2</sub> scavenging method than the standard (Ascorbic acid).

#### KEY WORDS

*Rosa damascena* Mill, DPPH, Hydroxyl, Nitric oxide, Ascorbic acid, Pylorus ligation and RanitidineHCl.

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#### INTRODUCTION<sup>1-3</sup>

*Rosa damascena* Mill is a medicinal as well as perfumery plant belonging to the family Rosaceae. It is cultivated in all over the world including Iran, Europe, Bulgaria, Turkey and India. It is an erect shrub, up to 2m tall, stems with large hooked prickles. Leaves are pinnate, leaflets 3-7. Flowers are red, pink and white in corymbs. Fruit ovoid or obovate, bright red when ripe. It has different pharmacological actions such as Antioxidant, Anti-

inflammatory, Antidiabetic, Anti-HIV, Laxative, Antiulcer, Cardio and Brain tonic.

## MATERIALS AND METHODS

### Collection of Plant material

The flowers of *Rosa damascena* Mill. were collected from Joharapuram village, Kurnool district, Andhra Pradesh, India. The flowers were authenticated by botanist Dr. P. Venu, Botanical Survey of India (BSI), Hyderabad, India. The voucher specimen was preserved in the Department of Pharmacognosy, C.E.S College of Pharmacy, Chinnatekur, Kurnool, Andhra Pradesh, India.

### Extraction Procedure<sup>4</sup>

The flowers were shade dried and powdered mechanically. The powder was extracted individually by using methanol, acetone and ethyl acetate as solvents. Methanol extract obtained was thick rose in color, sticky in nature and the percentage yield of the extract was found to be 35.66% w/w. Acetone extract obtained was rose incolor, sticky in nature and the percentage yield of the extract was found to be 24.83% w/w. Ethyl acetate extract obtained was light yellow in color, sticky in nature and the percentage yield of the extract was found to be 5.76% w/w. The methanol, acetone and ethyl acetate extracts were screened for phytochemical constituents and evaluated for *in-vitro* antioxidant and *in-vivo* antiulcer activities.

### Phytochemical Screening<sup>5,6</sup>

Extracts were screened for the presence of active principles such as flavonoids, phenols, steroids and triterpenoids, using following standard procedures.

### Determination of total phenolic content<sup>7</sup>

The total phenolic content was determined by Folin-Ciocalteu reagent method. The content of the phenols was determined as gallic acid equivalent. Stock solution (1mg/ml) of the methanol, acetone and ethyl acetate extracts were prepared in respective solvents. From the stock solutions 1ml of the extract was taken into a 25 ml volumetric flask. To this 10 ml of water and 1.5 ml of Folin-Ciocalteu reagent were added. The mixture was kept aside for 5 min, followed by addition of 4 ml of 20% Sodium Carbonate solution and volume was made up to 25

ml with distilled water. The mixture was kept aside for 30 min and the absorbance was recorded at 765nm using UV-Vis spectrophotometer. The total phenolic content was determined from extrapolation of calibration curve which was made by preparing gallic acid solution (50-150 µg/ml) in distilled water.

### Determination of total Flavonoid content<sup>8</sup>

The total flavonoid content was determined by aluminium chloride colorimetric method. The content of the flavonoids was determined as gallic acid equivalent. 1 ml of sample (1 mg/ml) was mixed with 3 ml of methanol, 0.2 ml of 10% aluminium chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water and remains at room temperature for 30 min. The absorbance of the reaction mixture was measured at 420nm with UV-Visible spectrophotometer. The total flavonoid content was determined from extrapolation of calibration curve which was made by preparing gallic acid solution (50-150 µg/ml) in distilled water.

### *In-vitro* antioxidant and radical scavenging activity

#### DPPH radical scavenging activity<sup>9</sup>

Ascorbic acid and dried extracts were weighed (10 mg each) and dissolved in 10 ml of methanol to get 1 mg/ml (1000 µg/ml) stock solutions separately. Lower concentrations of ascorbic acid and extracts (20, 40, 60, 80, 100 µg/ml respectively) were prepared by serially diluting stock solutions. The stable DPPH radical was used for determination of free radical-scavenging activity of the extracts. The 0.1 mM solution of DPPH in methanol (2.22 mg in 100 ml) was freshly prepared. Different concentrations of 3 ml of each extracts were added to 1 ml of methanolic solution of DPPH. After 30 min at room temperature, the absorbance was recorded at 517 nm. IC<sub>50</sub> values denote the concentration of sample, which is requiring scavenging 50% of DPPH free radicals. Radical scavenging activity was calculated by the following formula.

$$\begin{aligned} & \% \text{ of radical scavenging activity} \\ & = \left[ \left( \frac{A_c - A_s}{A_c} \right) \right] \times 100 \end{aligned}$$

Where A<sub>c</sub>: Absorbance of control, A<sub>s</sub>: Absorbance of standard/extract.

### Hydroxyl radical scavenging activity<sup>10</sup>

The stable Hydroxyl radical was used for determination of free radical-scavenging activity of the extracts. 2 ml of various (20, 40, 60, 80, 100 µg/ml) concentrations of extracts or standard were added to deoxy ribose (3 mM, 0.4 ml), ferric chloride (0.1 mM, 0.4 ml), EDTA (0.1 mM, 0.4 ml), ascorbic acid (0.1 mM, 0.4 ml) and hydrogen peroxide (2 mM, 0.4 ml) in phosphate buffer (pH, 7.4, 20 mM) and the reaction mixture was incubated at 37°C for 30 min after incubation, the reaction was stopped by adding ice cold trichloroacetic acid (0.2 ml, 15% w/v) and thiobarbituric acid (0.2 ml, 1% w/v), in 0.25 N HCl. The mixture was kept in a boiling water bath for 30 min, cooled and absorbance was measured at 532 nm. IC<sub>50</sub> values denote the concentration of sample, which is requiring scavenging 50% of hydroxyl free radicals. Radical scavenging activity was calculated by the following formula.

$$\% \text{ of radical scavenging activity} = \left[ \left( \frac{A_C - A_S}{A_C} \right) \right] \times 100$$

Where A<sub>C</sub>:Absorbance of control, A<sub>S</sub>: Absorbance of standard/extract.

### Nitric oxide radical scavenging activity<sup>11</sup>

The stable nitric oxide radical was used for determination of free radical-scavenging activity of the extracts. At different (20, 40, 60, 80, 100 µg/ml) concentrations 4 ml of extracts were added to 1 ml of sodium nitroprusside (25 mM), and incubated at 37°C for 2 hr. an aliquot (0.5 ml) of the incubation solution was removed and diluted with 0.3 ml Griess reagent (1% sulfanilamide in 5% H<sub>3</sub>PO<sub>4</sub> and 0.1% naphthylethylenediamine dihydrochloride equal amount), the absorbance was recorded at 517 nm. IC<sub>50</sub> values denote the concentration of sample, which is requiring scavenging 50% of nitric oxide free radicals. Radical scavenging activity was calculated by the following formula.

$$\% \text{ of radical scavenging activity} = \left[ \left( \frac{A_C - A_S}{A_C} \right) \right] \times 100$$

Where A<sub>C</sub>:Absorbance of control, A<sub>S</sub>: Absorbance of standard/extract.

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### Hydrogen peroxide scavenging activity<sup>12</sup>

The stable nitric oxide radical was used for determination of free radical-scavenging activity of the extracts. At different (20, 40, 60, 80, 100 µg/ml) concentrations 4 ml of extracts were added to 0.6 ml of 4 mM H<sub>2</sub>O<sub>2</sub> solution was prepared in phosphate buffer (0.1 M pH 7.4) and incubated for 10 min and the absorbance of the solution was recorded at 230 nm. IC<sub>50</sub> values denote the concentration of sample, which is requiring scavenging 50% of hydrogen peroxide free radicals. Radical scavenging activity was calculated by the following formula.

$$\% \text{ of radical scavenging activity} = \left[ \left( \frac{A_C - A_S}{A_C} \right) \right] \times 100$$

Where A<sub>C</sub>:Absorbance of control, A<sub>S</sub>:Absorbance of standard/extract.

### In-vivo anti-ulcer activity<sup>13-15</sup>

#### Pylorus ligation method

In this method Wistar rats of both sexes weighing between 150-250 gm were selected and divided into 9 groups and each group contains 6 animals. Animals fasted overnight. Group 1 serves as normal, group 2 received vehicle (saline), group 3 administered with Ranitidine (20 mg/kg) which serves as positive control, group 4 & 5 received methanol extract (250mg/kg and 500mg/kg), group 6 & 7 received acetone extract (250mg/kg and 500mg/kg), group 8 & 9 received ethyl acetate extract (250mg/kg and 500mg/kg) administered orally. After 30 minutes rats were lightly anesthetized by using chloroform and the abdomen was opened without damaging any blood supply. Then its pylorus was ligated. The abdomen was closed by suturing and the rats were allowed to recover for 4hr. After 4 hrs, the animals were anesthetized using chloroform and then euthanized by cervical dislocation. The abdomen was opened and a ligature was placed around the esophagus junction. The stomachs were removed and calculate the ulcer index and percentage inhibition.

#### Ethanol induced method

In this method wistar rats of both sexes weighing between 150-250 gm were selected and divided into 9 groups and each group contains 6 animals.

Animals fasted overnight. Group 1 serves as normal, group 2 received vehicle (saline), group 3 administered with Ranitidine (20 mg/kg) which serves as positive control, group 4 & 5 received methanol extract (250mg/kg and 500mg/kg), group 6 & 7 received acetone extract (250mg/kg and 500mg/kg), group 8 & 9 received ethyl acetate extract (250mg/kg and 500mg/kg) administered orally. One hour later, 1 ml of 80% ethanol was administered orally to each animal. Animals were sacrificed by cervical dislocation, one hour after ethanol administration, stomachs were isolated and cut open along the greater curvature and pinned on a soft board and finally calculated the ulcer index and percentage of inhibition.

Scoring of ulcers:

0 = Normal coloured stomach

0.5 = Red coloration

1 = Spot ulcer

1.5 = Haemorrhagic streaks

2 = Ulcers  $\geq 3$  but  $\leq 5$

3 = Ulcers  $> 5$

Calculation of ulcer index:

$$U_I = U_N + U_S + U_P \times 10^{-1}$$

$U_I$  = Ulcer index

$U_N$  = Average of no. of ulcers for animal

$U_S$  = Average of severity score

$U_P$  = % of animals with ulcers.

## RESULTS & DISCUSSION

### Phytochemical Screening

The methanol, acetone and ethyl acetate extracts of *Rosa damascena* Mill. was screened for its various phytoconstituents by standard chemical tests. Methanol, acetone extracts were found to contain flavonoids, phenols and steroids and ethyl acetate extract contains phenols, flavonoids and triterpenoids. The results were represented in Table No.1.

### Determination of total phenolic and total flavonoid Content of extracts

In the three extracts methanol extract contains high total phenolic and total flavonoid content and acetone, ethyl acetate extracts having less phenolic and flavonoid contents compared to methanol

extract. The results were represented Table No.2, Figure No.1, 2.

### In-vitro antioxidant studies

#### DPPH radical scavenging activity

This assay showed the abilities of the extract and standard ascorbic acid to scavenge DPPH radical at concentration range of 20-100 $\mu$ g/ml in a concentration dependent manner. Decrease in absorbance with increase in concentration indicates a concentration response relationship in DPPH scavenging activity of extracts. The methanol extract of *Rosa damascena* Mill. Shown significant DPPH scavenging effect with an  $IC_{50}$  value of 14 $\mu$ g/ml and the percentage inhibition is 82.72% compared to ascorbic acid. The results were represented in Table No.3, Figure No. 3.

#### Hydroxyl radical scavenging activity of extracts

The degradation of hydroxyl radical scavenging was significantly decreased by *Rosa damascena* Mill. and ascorbic acid at concentration range of 20-100  $\mu$ g/ml in concentration dependent manner, proving the significant hydroxyl radical scavenging activity of extracts. The methanol extract showed significant antioxidant activity ( $IC_{50}$ =18  $\mu$ g/ml and percentage inhibition value is 78.73%) compared to standard ( $IC_{50}$ =14.5  $\mu$ g/ml and percentage inhibition value is 79.33%). The results were represented in Table No.4, Figure. No.4.

#### Nitric oxide radical scavenging activity of extracts

The extracts showed a significant nitric oxide scavenging activity between concentration range of 20 to 100  $\mu$ g/ml in a concentration dependent manner. Acetone extract of *Rosa damascena* Mill. showed significant nitric oxide scavenging activity, the ( $IC_{50}$ ) value of the acetone extract was found to be 44  $\mu$ g/ml and percentage inhibition value is 78.21% and standard ascorbic acid ( $IC_{50}$ ) value was 40  $\mu$ g/ml and percentage inhibition value is 83.56%. The results were represented in Table No. 5, Figure No. 5.

#### Hydrogen peroxide scavenging activity of extracts

The extracts was able to neutralize  $H_2O_2$  in a concentration dependent manner at a concentration range of 20-100  $\mu$ g/ml. Ethyl acetate ( $IC_{50}$ =15  $\mu$ g/ml and the percentage inhibition value is 82.72%)

extract showed better antioxidant activity than the standard ascorbic acid ( $IC_{50}=41 \mu\text{g/ml}$  and the percentage inhibition value is 81.86%). The results were represented in Table No.6, Figure No. 6.

**Biological activity (in-vivo anti-ulcer activity)**

**Pylorus ligation method**

In this method all extracts showed the antiulcer activity at the doses of 500 mg/kg when compared to standard (Ranitidine). Methanol extract showed a percentage inhibition of 72.48% compared to standard (84.10%) at the dose level of 500 mg/kg, acetone and ethyl acetate extracts showed percentage inhibition of 65.89% and 60.07% respectively at the dose level of 500 mg/kg. The order of percentage inhibitions showed by the extracts is

Methanol>Acetone>Ethyl acetate. The results were represented in Table No. 7, Figure No. 7.

**Ethanol induced method**

In this method all extracts showed the antiulcer activity at the doses of 500 mg/kg when compared to standard (Ranitidine). Methanol extract showed a percentage inhibition of 61.84% compared to standard (72.76%) at the dose level of 500 mg/kg, acetone and ethyl acetate extracts showed percentage inhibition of 47.04% and 57.55% respectively at the dose level of 500 mg/kg. The order of percentage inhibitions showed by the extracts is Methanol>Ethyl acetate>Acetone. The results were represented in Table No. 8.

**Table No.1: Phytochemical screening of the methanol, acetone and ethyl acetate extracts of *Rosa damascena* Mill**

S.No.	Plant constituents	Inference		
		Methanol extract	Acetone extract	Ethyl acetate extract
1	Alkaloids	-	-	-
2	Carbohydrates	-	-	-
3	Flavonoids	+	+	+
4	Phenols	+	+	+
5	Steroids	+	+	-
6	Triterpenoids	-	-	+

**Table No.2: Total phenolic and total flavonoid contents in methanol, acetone and ethyl acetate extracts of *Rosa damascene* Mill**

S. No	Extract	Total phenolic content (Mean±SEM) (GAE $\mu\text{g/g}$ of dry material)	Total flavonoid content (Mean±SEM) (GAE $\mu\text{g/g}$ of dry material)
1	Methanol extract	100.5±0.402	116.25±0.465
2	Acetone extract	89.5±0.358	58.2±0.233
3	Ethyl acetate extract	73±0.292	43.5±0.174

**Table No.3: DPPH radical scavenging activity of methanol, acetone and ethyl acetate extracts of *Rosa damascena* Mill**

S. No	Sample	Concentration	Absorbance (Mean±SEM)	Percentage inhibition (Mean±SEM)	IC <sub>50</sub>
1	Methanol extract	20µg/ml	0.058±0.003	64.41±1.623	14µg/ml
		40µg/ml	0.047±0.001	71.16±0.710	
		60µg/ml	0.051±0.002	74.84±7.110	
		80µg/ml	0.037±0.003	77.09±1.781	
		100µg/ml	0.020±0.001	82.72±0.707	
2	Acetone extract	20µg/ml	0.077±0.004	52.75±0.707	19µg/ml
		40µg/ml	0.059±0.002	63.80±1.275	
		60µg/ml	0.055±0.002	66.25±1.062	
		80µg/ml	0.047±0.002	70.95±1.082	
		100µg/ml	0.044±0.006	73.00±3.418	
3	Ethyl acetate extract	20µg/ml	0.057±0.002	65.02±0.938	15µg/ml
		40µg/ml	0.054±0.002	66.87±1.062	
		60µg/ml	0.054±0.002	67.07±1.139	
		80µg/ml	0.041±0.001	74.84±1.062	
		100µg/ml	0.040±0.002	75.72±1.242	
4	Ascorbic acid	20µg/ml	0.056±0.003	65.84±1.816	12µg/ml
		40µg/ml	0.059±0.005	63.59±2.861	
		60µg/ml	0.048±0.002	70.01±0.775	
		80µg/ml	0.039±0.002	76.07±1.062	
		100µg/ml	0.057±0.002	89.63±1.005	

**Table No.4: Hydroxyl radical scavenging activity of methanol, acetone and ethyl acetate extracts of *Rosa damascena* Mill**

S. No	Sample	Concentration	Absorbance (Mean±SEM)	Percentage inhibition (Mean±SEM)	IC <sub>50</sub>
1	Methanol extract	20 µg/ml	0.187±0.001	52.29±0.294	18 µg/ml
		40 µg/ml	0.176±0.003	55.10±0.779	
		60 µg/ml	0.162±0.001	58.58±0.372	
		80 µg/ml	0.122±0.006	68.95±1.532	
		100 µg/ml	0.092±0.005	78.73±3.351	
2	Acetone extract	20 µg/ml	0.171±0.002	56.29±0.450	18.5 µg/ml
		40 µg/ml	0.154±0.002	60.71±0.589	
		60 µg/ml	0.142±0.002	63.77±0.442	
		80 µg/ml	0.118±0.001	69.97±0.372	
		100 µg/ml	0.095±0.003	75.76±0.739	
3	Ethyl acetate	20 µg/ml	0.174±0.007	55.52±1.863	
		40 µg/ml	0.175±0.005	55.35±1.170	

	<b>extract</b>	60 µg/ml	0.150±0.002	61.64±0.615	19 µg/ml
		80 µg/ml	0.143±0.002	63.60±0.456	
		100 µg/ml	0.135±0.002	65.55±0.532	
4	<b>Ascorbic acid</b>	20 µg/ml	0.142±0.002	64.93±0.450	14.5 µg/ml
		40 µg/ml	0.117±0.001	70.23±0.226	
		60 µg/ml	0.106±0.003	72.86±0.756	
		80 µg/ml	0.090±0.004	76.95±1.001	
		100 µg/ml	0.081±0.002	79.33±0.388	

**Table No.5: Nitric oxide radical scavenging activity of methanol, acetone and ethyl acetate extracts of *Rosa damascena* Mill**

S. No	Sample	Concentration	Absorbance (Mean±SEM)	Percentage inhibition (Mean±SEM)	IC <sub>50</sub>
1	<b>Methanol extract</b>	20µg/ml	0.120±0.001	44.16±0.55	44µg/ml
		40µg/ml	0.113±0.003	50.07±1.351	
		60µg/ml	0.087±0.001	59.22±0.677	
		80µg/ml	0.070±0.003	63.87±0.674	
		100µg/ml	0.057±0.001	73.33±0.674	
2	<b>Acetone extract</b>	20µg/ml	0.114±0.001	46.66±0.674	38µg/ml
		40µg/ml	0.111±0.001	48.36±0.805	
		60µg/ml	0.091±0.001	57.51±0.677	
		80µg/ml	0.074±0.002	65.26±1.085	
		100µg/ml	0.057±0.002	78.21±1.351	
3	<b>Ethyl acetate extract</b>	20µg/ml	0.118±0.003	44.95±1.480	48µg/ml
		40µg/ml	0.114±0.001	46.81±0.558	
		60µg/ml	0.093±0.002	56.89±0.820	
		80µg/ml	0.078±0.001	63.87±0.674	
		100µg/ml	0.059±0.003	72.55±1.493	
4	<b>Ascorbic acid</b>	20µg/ml	0.120±0.002	44.18±0.97	40µg/ml
		40µg/ml	0.090±0.005	56.42±1.528	
		60µg/ml	0.067±0.001	68.74±0.544	
		80µg/ml	0.058±0.002	73.01±0.805	
		100µg/ml	0.035±0.001	83.56±0.677	

**Table No.6: H<sub>2</sub>O<sub>2</sub> radical scavenging activity of methanol, acetone and ethyl acetate extracts of *Rosa damascena* Mill**

S. No	Sample	Concentration	Absorbance (Mean ±SEM)	Percentage inhibition (Mean±SEM)	IC <sub>50</sub>
1	Methanol extract	20µg/ml	0.128±0.001	37.41±0.712	80µg/ml
		40µg/ml	0.121±0.006	40.68±2.786	
		60µg/ml	0.119±0.003	41.49±1.276	
		80µg/ml	0.103±0.002	49.67±0.867	
		100µg/ml	0.074±0.003	63.72±1.497	
2	Acetone extract	20µg/ml	0.121±0.002	40.51±0.909	53µg/ml
		40µg/ml	0.112±0.001	44.92±0.712	
		60µg/ml	0.095±0.003	53.59±1.558	
		80µg/ml	0.083±0.002	59.31±0.748	
		100µg/ml	0.056±0.002	72.70±1.143	
3	Ethyl acetate extract	20µg/ml	0.115±0.002	64.41±1.623	15µg/ml
		40µg/ml	0.105±0.002	71.16±0.710	
		60µg/ml	0.097±0.003	74.84±7.110	
		80µg/ml	0.080±0.001	77.09±1.781	
		100µg/ml	0.064±0.003	82.72±0.707	
4	Ascorbic acid	20µg/ml	0.113±0.001	44.60±0.566	41 µg/ml
		40µg/ml	0.096±0.001	52.94±0.566	
		60µg/ml	0.056±0.004	72.38±1.928	
		80µg/ml	0.049±0.003	76.15±1.278	
		100µg/ml	0.037±0.002	81.86±1.020	

**Table No.7: In-vivo antiulcer activity of methanol, acetone and ethyl acetate extracts of *Rosa damascena* Mill. by pylorus ligation method**

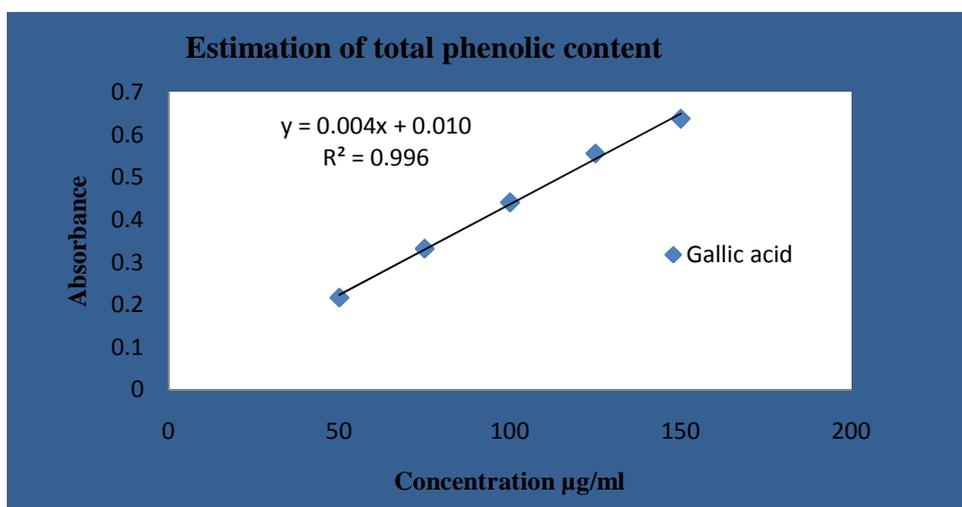
S. No	Groups	Treatment	Ulcer index	% inhibition	
1	Group-I	Normal	1.8±0.03	-	
2	Group-II	Control	25.8±1.53	-	
3	Group-III	Standard	4.1±0.28***	84.10	
4	Group-IV	Methanol extract (T <sub>1</sub> )	250mg/kg	15.6±0.98	39.53
5	Group-V		500mg/kg	7.1±1.83***	72.48
6	Group-VI	Acetone extract (T <sub>2</sub> )	250mg/kg	16.3±1.19	36.84
7	Group-VII		500mg/kg	8.8±1.03**	65.89
8	Group-VIII	Ethyl acetate extract (T <sub>3</sub> )	250mg/kg	19.3±1.63	25.19
9	Group-IX		500mg/kg	10.3±1.23*	60.07

Significance controls \*P<0.05, \*\*P<0.01, \*\*\*P<0.001 compare to the ANOVA T test, (n=6)

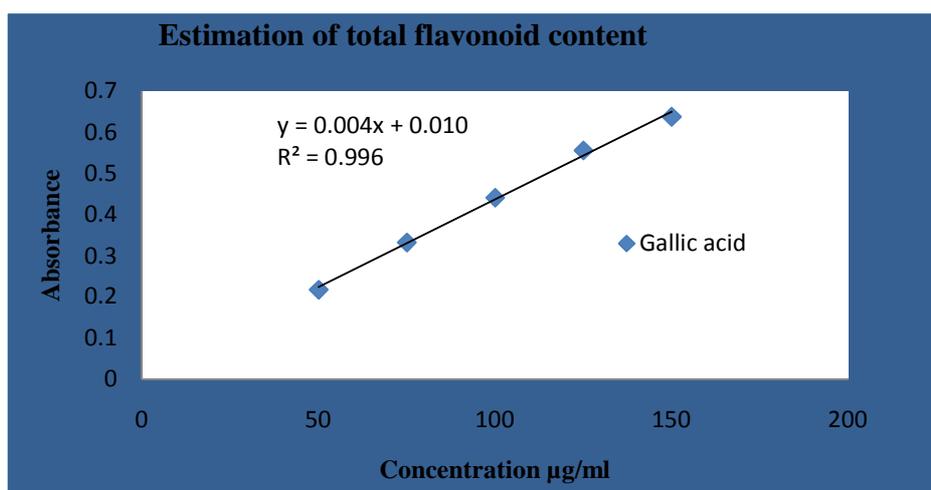
**Table 8: In-vivo antiulcer activity of methanol, acetone and ethyl acetate petals extracts of *Rosa damascena* Mill. by ethanol induced method.**

S. No	Groups	Treatment	Ulcer index	% inhibition
1	Group-I	Normal	1.8±0.03	-
2	Group-II	Control	19.13±2.12	-
3	Group-III	Standard	5.21±1.31***	72.76
4	Group-IV	Methanol extract (T <sub>1</sub> )	250mg/kg	12.63±1.38
5	Group-V		500mg/kg	7.3±0.98***
6	Group-VI	Acetone extract (T <sub>2</sub> )	250mg/kg	13.13±1.63
7	Group-VII		500mg/kg	10.13±1.05**
8	Group-VIII	Ethyl acetate extract (T <sub>3</sub> )	250mg/kg	14.5±1.38
9	Group-IX		500mg/kg	8.12±1.62*

Significance controls \*P<0.05, \*\*P<0.01, \*\*\*P<0.001 compare to the ANOVA T test, (n=6).



**Figure No.1: Standard calibration curve of gallic acid for estimation of total phenolic content**



**Figure No.2: Standard calibration curve of gallic acid for estimation of total flavonoid content**

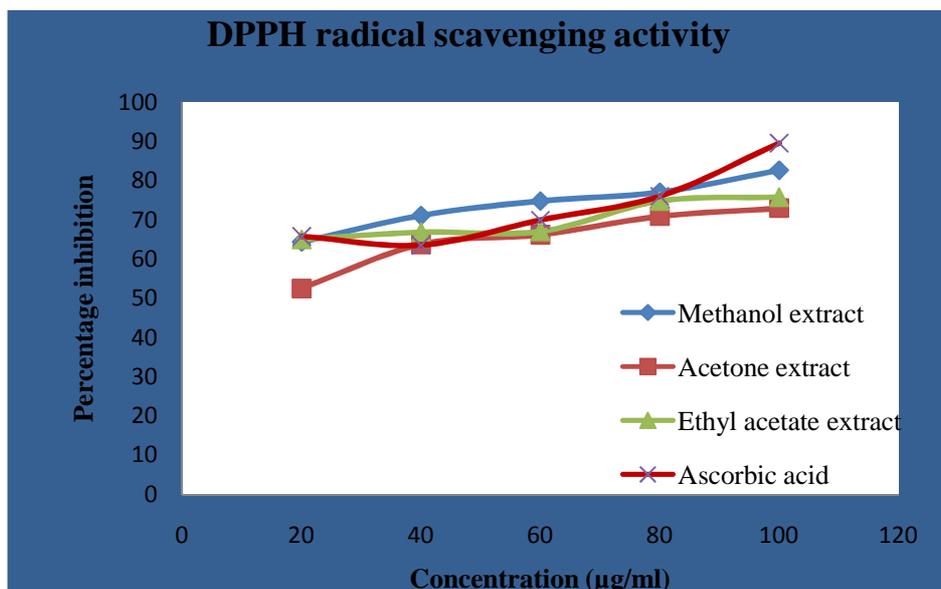


Figure No.3: DPPH radical scavenging activity of methanol, acetone and ethyl acetate extracts of *Rosa damascena* Mill.  
Values are expressed as the Mean±SEM, (n=3)

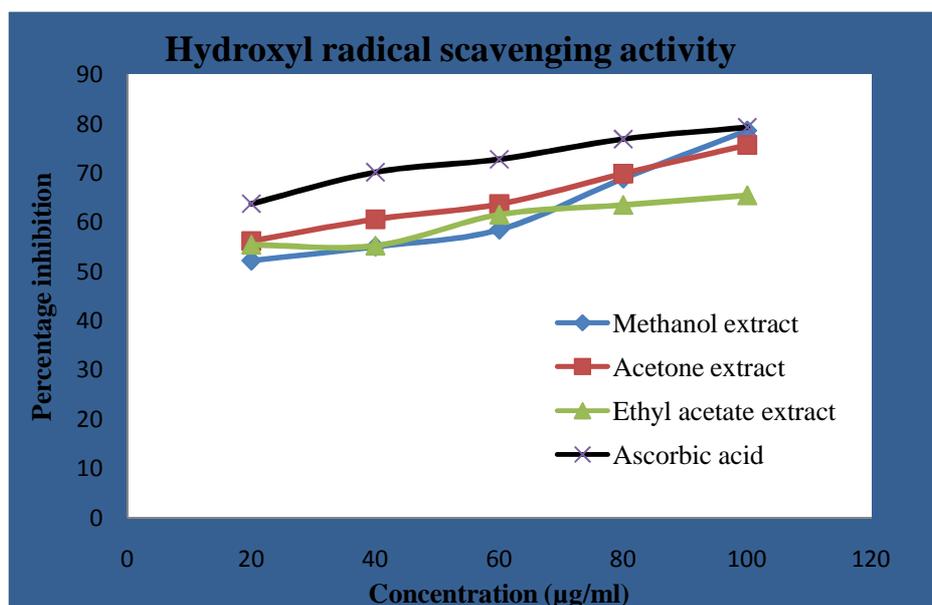
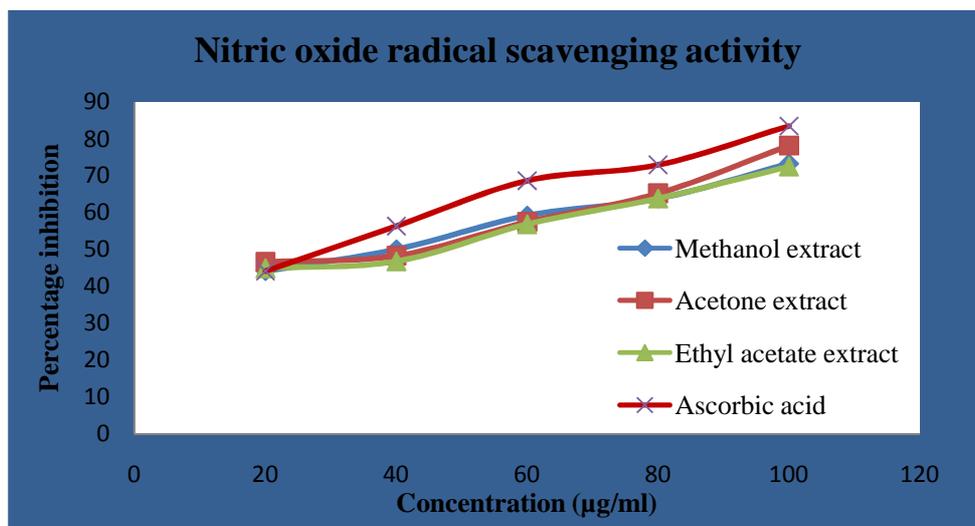
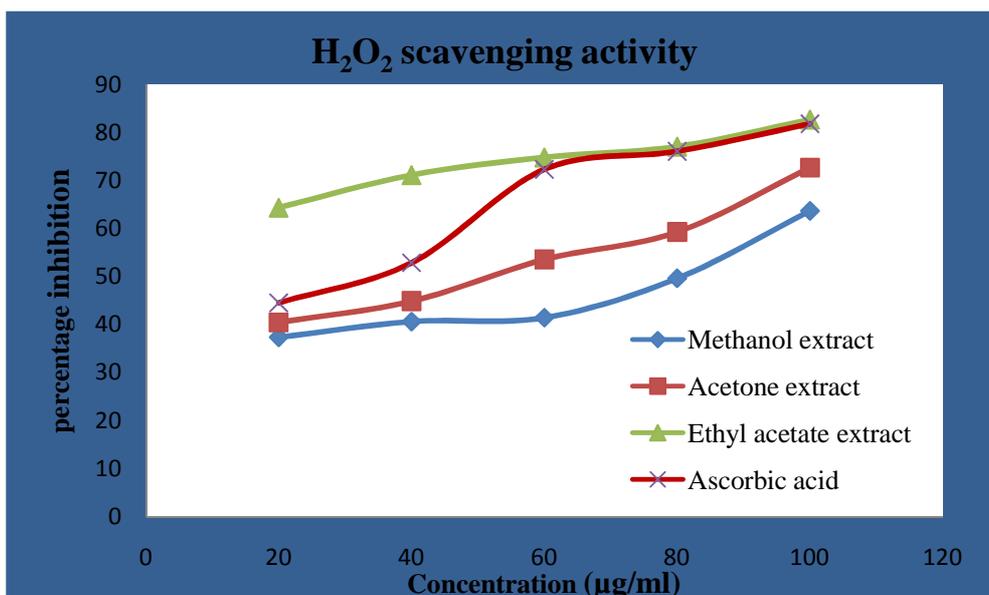


Figure N0.4: Hydroxyl radical scavenging activity of methanol, acetone and ethyl acetate extracts of *Rosa damascena* Mill.  
Values are expressed as the Mean±SEM, (n=3)



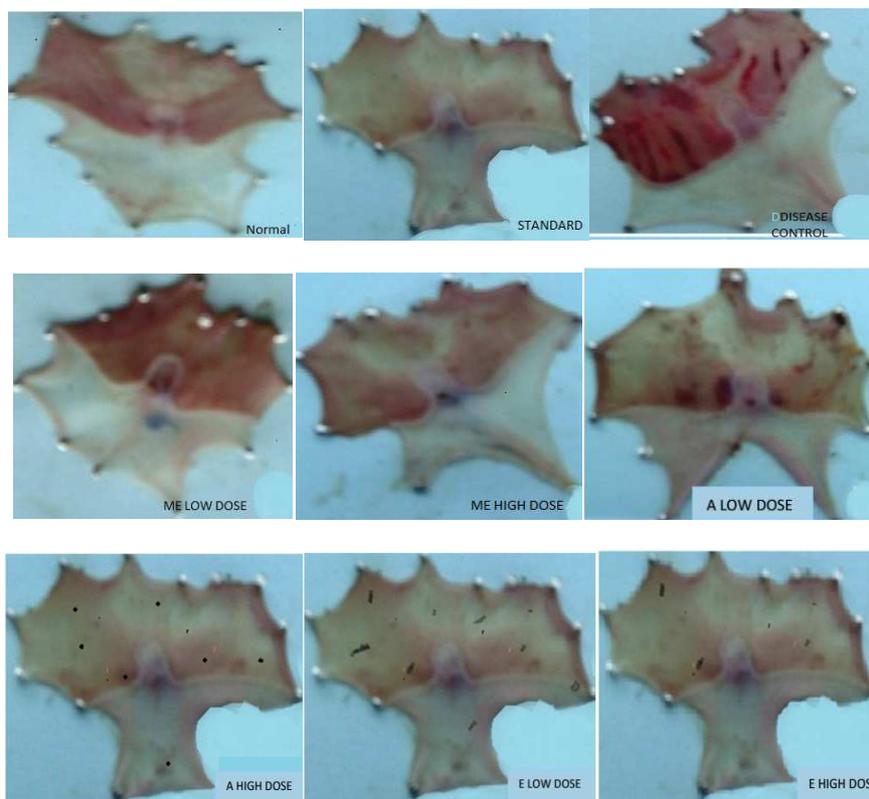
**Figure 5: Nitric oxide radical scavenging activity of methanol, acetone, and ethyl acetate extracts of *Rosa damascena* Mill**

Values are expressed as the Mean±SEM, (n=3)



**Figure 6: H<sub>2</sub>O<sub>2</sub> scavenging activity of methanol, acetone and ethyl acetate extracts of *Rosa damascena* Mill.**

Values are expressed as the Mean±SEM, (n=3).



**Figure No.7: Diagrammatic representation of antiulcer activity *Rosa damascena* Mill. by pylorus ligation method.**

## CONCLUSION

Flowers were collected and authenticated, shade dried, powdered and extracted by using methanol, acetone and ethyl acetate solvents individually in Soxhlet apparatus. The methanol, acetone and ethyl acetate petals extracts of *Rosa damascena* Mill. was evaluated for *in-vitro* antioxidant studies by using DPPH, Nitric oxide, Hydroxyl and H<sub>2</sub>O<sub>2</sub> radical scavenging methods and screen for antiulcer activity. In antioxidant studies methanol extract showed significant antioxidant activity in DPPH and Hydroxyl radical scavenging method in nitric oxide method acetone extract possess significant activity compared to standard and in ethyl acetate extract shows excellent activity than the standard in H<sub>2</sub>O<sub>2</sub> scavenging activity. The methanol extract of petals of *Rosa damascena* Mill. showed significant antiulcer activity in pylorus ligation and ethanol induced method. Hence we suggest that the petals

of the plant can be viewed as the potential source of natural antioxidant and antiulcer agents can afford precious functional components.

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