

## Phytochemical Analysis, *In-Vitro* Antioxidant and Antimicrobial Activities of Flower Petals of *Rosa damascena*

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### ABSTRACT

*Rosa damascena* commonly called as Rose is one of the most important plant for the pharmaceutical, flavour and fragrance industries. Rose petals extract and rose water is commonly used for skin enhancement by the cosmetics industry. The objective of the present study was to evaluate the antioxidant and antibacterial activity of aqueous and methanol extracts of *Rosa damascena* flower petals. Individual aqueous and methanol extract were prepared by Soxhlet extraction method. Phytochemical analysis of the extracts showed the presence of carbohydrates, proteins, tannins, phenols and alkaloids. The total phenolic content and total flavonoid content was also determined. The hydrogen donating ability of the extracts were measured in the presence of 1,1- diphenyl- 2- picryl-hydrazyl (DPPH) radical. The IC<sub>50</sub> values of aqueous and methanol extract were 8.30µg/ml and 4.85 µg/ml respectively by DPPH method and 70.90µg/ml and 47.18µg/ml respectively by Griess assay method. The IC<sub>50</sub> values were comparable with standard ascorbic acid. Antibacterial activity of the extracts was determined by the agar diffusion method against 9 species of bacteria and fungi: *Staphylococcus aureus*, *Sterptococcus pyogens*, *Clostridium perfringens*, *Pseudomonas aeroginosa*, *Escherichia coli*, *Klebsiella pneumonia*, *Klebsiella aerogens*, *Candida albicans* and *Aspergillus niger*. Both extracts were found to be effective against all the bacteria except *Aspergillus niger*. Methanol extract showed good antimicrobial activity at 40 mg whereas aqueous extract showed good antimicrobial activity at 80 mg when compared with standard Chloramphenicol and Fluconazole. The presence of flavanoids and phenolic compounds in the *Rosa damascena* flower extracts may have contributed to the antioxidant and antibacterial activities.

**Key words:** *Rosa damascena*, Phytochemical analysis, Antioxidant activity, Antibacterial activity.

### INTRODUCTION

Biochemical reactions in the body generate reactive oxygen species which can damage important biomolecules<sup>1</sup>. These reactive oxygen species are known to be the major cause of various chronic and degenerative diseases<sup>2</sup>. The harmful action of the free radicals can be blocked by antioxidants which scavenge the free radicals and nullify their damaging effect on cellular constituents. Natural antioxidants from plants have been shown to increase the antioxidant capacity of the plasma and reduce the risk of certain diseases such as cancer, heart diseases and stroke<sup>3</sup>.

Currently used synthetic antioxidants and antimicrobials have been shown to have harmful side effects such as development of resistance<sup>1</sup>. Medicinal plants are potential sources of antimicrobial and antioxidant compounds. it is therefore essential to thoroughly investigate their composition and activity and thus validate their use<sup>4</sup>.

The importance of medicinal plants as a source of active drugs emerged from the chemical profile that produces a clear physiological action on the biological system. Flavonoids, alkaloids, tannins and phenolic compounds have been established as the most important bioactive compounds of plants. Plant tannins and flavonoids have

been reported to have antimicrobial effects and many biological activities<sup>5</sup>. *Rosa damascena* Mill (Rosaceae) is widely cultivated ornamental plant. The flower petal of this plant is reported to have blood purifying properties<sup>6</sup>. In ancient medical books several therapeutic effects of this plant, such as treatment of abdominal and chest pain are reported<sup>7</sup>. Its flowers are reported to have astringent, analgesic, anti-inflammatory, antidepressant, antibacterial, diuretic and anti-HIV activity and used in folk medicine as a mild laxative<sup>8</sup>. Rose flower oil and rose flower extracts have been studied for a number of health beneficial effects including antibacterial activity, antioxidant activity, anti-infective and anti-inflammatory properties and relaxant effect on tracheal chains<sup>9</sup>. The present study was undertaken to evaluate the phytochemical analysis, *in-vitro* antioxidant activity and antimicrobial activity of *Rosa damascena* flower petal extracts.

### MATERIALS AND METHODS

#### *Plant Material*

The air dried powder of *Rosa damascena* flower petals was provided by Amsar Pvt Ltd.

#### *Test Organisms*

Table 1: Qualitative analysis of extracts of *Rosa damascena* flower petals

Test	Aqueous extract	Methanol extract
Carbohydrates	+	+
Proteins/amino acids	+	+
Glycosides	-	+
Saponins	+	-
Alkaloids	-	+
Tannins	+	+
Flavonoids	-	+
Steroids	-	-
Gums/mucilage	-	-
Fixed oil and fats	-	-

(+) = indicates presence, (-) = indicates absence

Table 2: Total Phenolic and total flavanoid content of extracts of *Rosa damascena*

S. no	<i>Rosa damascena</i> extract	Total phenolic content (mg GAE/g) of extract	Total flavanoid content (mg RE/g) of extract/
1.	Aqueous extract	49.01 ± 0.167	55.09 ± 0.31
2.	Methanol extract	69.17 ± 0.167	76.28 ± 0.29

Table 3: Antioxidant activity of *R. damascena* flower extract and ascorbic acid

Sample	IC50 (µg/ml)			
	DPPH radical scavenging activity	Free Nitric oxide scavenging activity	Nitric oxide scavenging activity	
Aqueous extract	8.308		70.904	
Methanol extract	4.85		47.181	
Ascorbic acid (standard)	11.181		27.117	

Test organisms used for the study were obtained from National Chemical laboratory, Pune, Maharashtra.

#### Reagents and Chemicals

All reagents and chemicals used in the experiments were of analytical grade and obtained from Sigma Aldrich (USA). Solvents used for extraction of plant material were of analytical grade. Ascorbic acid, DPPH (1,1-diphenyl-2-picrylhydrazyl), Sodium nitroprusside, Sulphanilic acid and  $\alpha$ -naphthyl-ethylene diamine were purchased from Sigma Aldrich (USA).

#### Extraction of Plant Material

The air dried powder of *Rosa damascena* flower petals was subjected to continuous hot extraction with methanol for 18 hours. Aqueous extract was prepared by refluxing the plant material with water for 8 hours. The extracts were concentrated using rotary vacuum evaporator below 40°C. The dried extracts were stored in vacuum desiccator for further use.

#### Phytochemical Analysis<sup>10, 11</sup>

Preliminary qualitative phytochemical screening of the aqueous and methanol extracts were carried out to know the different phytoconstituents present in flower petals of *R. damascena* as per the standard procedure.

#### Total Phenolic content<sup>12</sup>

The amount of total phenolic and tannins in the plant extract was determined calorimetrically with the Folin-Ciocalteu reagent (FCR). The reaction mixture contained 50µl of the extract (1mg/ml) in methanol, 250µl of FCR, 750µl of sodium carbonate solution. The volume was made upto 5 ml with distill water and was incubated in dark under ambient conditions for 2 hours to complete the reaction. In the control tube, the extract volume was replaced by methanol.

The absorbance of the resulting solution was measured at 760nm in a UV spectrophotometer. The concentration of total phenolics and tannins was expressed as mg of gallic acid equivalents (GAE) per g of dried extract, using a standard curve of gallic acid. All the measurements were carried out in triplicates.

Total phenolic content was calculated using the following formula:

$C = c \times V / m$ , where

$C$  = total content of phenolic compounds in mg/g plant extract in GAE or mg GAE/g extract

$c$  = the concentration of gallic acid established from the calibration curve in mg/ml

$V$  = the volume of extract in ml;  $m$  = the weight of plant extract in g

#### Total Flavanoid content<sup>13</sup>

The flavonoids content in the plant extract was determined by aluminium chloride method using rutin as a reference compound. The 100µL of plant extract (10mg/ml) in methanol was mixed 100µL with 20% aluminium trichloride in methanol and a drop of acetic acid, and then diluted with methanol to 5ml. The absorption at 415nm was read after 40 minutes. Blank consists of 100µL of extract, a drop of acetic acid and adjusted to 5mL with methanol. The absorption of standard rutin solution (0.5mg/ml) in methanol was measured under the same conditions. All measurements were carried out in triplicates. The amount of flavonoids in the extract in rutin equivalents (RE) was calculated using following formula-

$$X = (A_o \cdot m_o) / (A \cdot m)$$

Where,  $X$  is the flavanoid content of extract in mg/g extract in RE or mg RE/g extract,  $A$  is the absorption of plant extract solution,  $A_o$  is the absorption of standard rutin solution,  $m$  is the weight of extract in mg and  $m_o$  is the weight of rutin in the solution in mg.

#### Antioxidant Activity

##### DPPH Free radical scavenging activity<sup>14, 15</sup>

The free radical scavenging activity of the extracts were measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH (1,1-diphenyl-2-picryl-hydrazyl). 0.1 mM solution of DPPH in methanol was prepared and 1.0 ml of this solution was added to 1.0 ml of dilutions of extracts at different concentrations (2-20 µg/ml). Thirty minutes later, the absorbance was measured at 517 nm. Ascorbic acid was

Table 4: Antimicrobial activity of extracts *Rosa damascena* by Cup plate method

Organisms	Zone of Inhibition (mm)						
	Chloramphenicol (100µg) (µg/ well)	Aqueous Extract (mg/ well)			Methanol extract (mg/ well)		
		20 mg	40 mg	80 mg	20 mg	40 mg	80 mg
<i>S.aureus</i>	7 ± 0.13	2.1 ± 0.1	3.9 ± 0.13	5.9 ± 0.2	4.9 ± 0.13	6.9 ± 0.13	8.8 ± 0.13
<i>E. coli</i>	7.7 ± 0.13	-	3.3 ± 0.17	7.2 ± 0.1	2 ± 0.1	6.4 ± 0.2	11.2 ± 0.1
<i>K. pneumoniae</i>	13.7 ± 0.13	6.5 ± 0.1	10.2 ± 0.1	13.2 ± 0.1	8.4 ± 0.1	14.7 ± 0.13	16.4 ± 0.2
<i>K. aerogens</i>	8.7 ± 0.13	2.2 ± 0.1	4.1 ± 0.1	7.7 ± 0.13	3.5 ± 0.1	8.3 ± 0.17	12.1 ± 0.1
<i>P. aeruginosa</i>	9.7 ± 0.13	-	-	-	-	8.8 ± 0.13	13.6 ± 0.2
<i>S.pyogens</i>	8.2 ± 0.1	-	3.2 ± 0.1	7.8 ± 0.13	2.3 ± 0.17	6.6 ± 0.2	10.2 ± 0.2
<i>C.perfringens</i>	13.3 ± 0.17	-	10.1 ± 0.1	12.6 ± 0.2	7.5 ± 0.1	13.2 ± 0.1	16.2 ± 0.2
	Fluconazole						
<i>C. albicans</i>	15.3 ± 0.17	-	-	-	8.3 ± 0.17	14.9 ± 0.20	21 ± 0.23
<i>A. niger</i>					-	-	-

used as standard. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH Scavenged (\%)} = (A_{\text{cont}} - A_{\text{test}}) / A_{\text{cont}} \times 100$$

where  $A_{\text{cont}}$  is the absorbance of the control reaction and  $A_{\text{test}}$  is the absorbance in the presence of extract.

#### Nitric oxide radical scavenging activity<sup>16</sup>

Nitric oxide radical inhibition was estimated by use of Griess assay. Sodium nitroprusside (10 mM, 1.5 ml) in phosphate –buffered saline (PBS) was mixed with 0.5 ml of different concentrations (10- 100 µg/ml) of the extract dissolved in the suitable solvent systems and incubated at 25°C for 150 min. The sample mixtures were then reacted with 1 ml Griess reagent for 15 mins (1% sulphanilamide, 2%  $\text{H}_3\text{PO}_4$  and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylenediamine was read at 546 nm with reference to the absorbance of standard solutions of ascorbic acid, treated in the same way with Griess reagent.

The % of NO scavenging activity is calculated as follows: Scavenging Effect (%) =  $(A_{\text{cont}} - A_{\text{test}}) / A_{\text{cont}} \times 100$

Where,  $A_{\text{cont}}$  is the absorbance of the control reaction and  $A_{\text{test}}$  is the absorbance in the presence of the sample of the extracts.

#### Anti- Bacterial Activity

Two techniques were used to test the anti- microbial activity of *Rosa damascena* extracts; ditch plate technique and agar well diffusion technique against the reference bacterial and fungal strains.

#### Strains tested

Gram positive- *Staphylococcus aureus* (NCIM- 5022), *Sterptococcus pyogens* (NCIM- 2608), *Clostridium perfringens* (NCIM- 2677).

Gram negative- *Pseudomonas aeroginosa* (NCIM- 2200), *Escherichia coli* (NCIM- 2065), *Klebsiella pneumonia* (NCIM- 5082), *Klebsiella aerogenes* (NCIM- 2239)

Fungi- *Candida albicans* (NCIM- 3471), *Aspergillus niger* (NCIM- 1196)

Authentic pure cultures of bacteria were cultivated in nutrient broth (NB) or nutrient agar at  $37 \pm 0.2^\circ\text{C}$ .

Fungi and yeasts were cultured on sabouraud dextrose agar at  $28 \pm 0.2^\circ\text{C}$ . The cultures of bacteria and fungi were maintained in their appropriate agar slants at  $4^\circ\text{C}$  throughout the study and used as stock cultures. Microbial cultures were suspended in a saline solution (0.85% NaCl) and adjusted to a turbidity of 0.5 MacFarland standards (108 CFU/ml).

#### Ditch plate method<sup>17</sup>

Ditch plate method is used for primary screening of extracts against various organisms. The antibacterial susceptibility of each organism to the test extracts is tested in this method. In this technique 30ml of molten agar (nutrient agar / Sabouraud dextrose agar) was poured into a sterile petri plate. The molten agar was allowed to set and harden at room temperature. Ditch (1cm × 3 cm) was made in the agar plate using a sterile scalpal. Solution of each extract (1ml solution containing 50mg of extract) was placed in ditch. loopful of inoculums of each organism (gram positive, gram negative and fungi) were streaked across the agar at right angle to the ditch. All the petri plates containing bacterial cultures were incubated at  $37^\circ\text{C}$  for 24 hours. Plates containing fungal cultures were incubated at  $28^\circ\text{C}$  for 48 hours.

#### Agar well diffusion method<sup>18</sup>

The extracts were then evaluated for antimicrobial activity using agar well diffusion method. Nutrient agar plates were seeded with 1 ml of bacterial suspension and Sabouroud dextrose agar plates with fungal strain (equivalent to  $10^6\text{cfu/ml}$ ). The seeded plates were allowed to set. A sterile cork borer of 11 mm diameter was then used to cut equidistant wells on the surface of the agar. The wells were filled with 0.4 ml solution of each extract at various concentrations (20, 40 and 80 mg/well). The plates were incubated at  $37^\circ\text{C}$  for 24 h and  $28^\circ\text{C}$  for 48 hours after which the diameter of zones of inhibition were measured. Chloramphenicol (100µg) and Fluconazole (15µg) were used as positive control. All the experiments were done in triplicates.

#### Statistical Analysis

All experiments were carried out in triplicates. The values are expressed as mean ± SEM. Students paired t-test was applied to determine statistical significance.  $p \leq 0.05$  was considered significant.

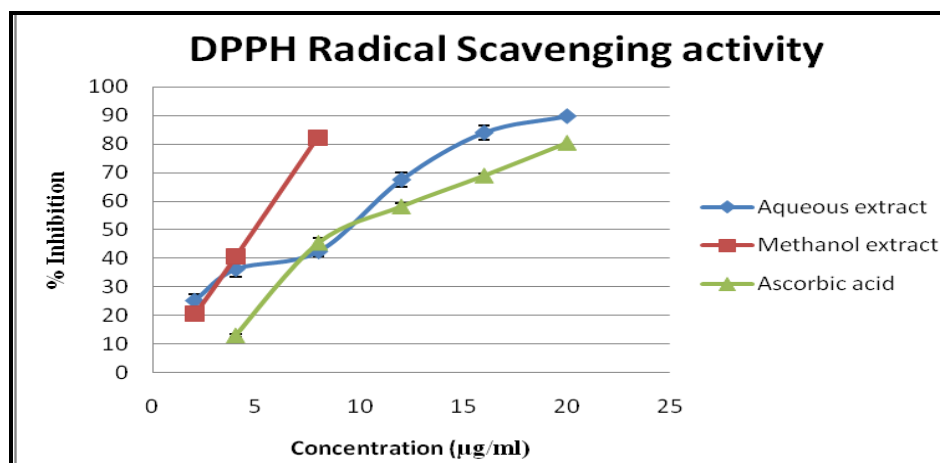


Fig.1 DPPH free radical scavenging activity of *R. damascena* flower extracts and ascorbic acid

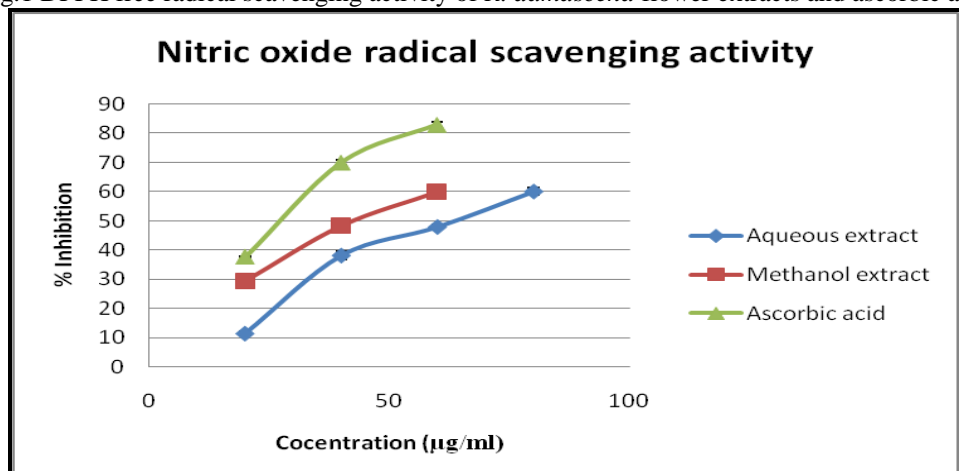


Fig.2 Nitric Oxide radical scavenging activity of *R. damascena* flower extracts and ascorbic acid

## RESULT AND DISCUSSION

### Phytochemical Analysis

The qualitative phytochemical analysis of aqueous and methanol extracts revealed the presence of carbohydrates, proteins, glycosides, alkaloids, tannins, phenols and saponins (Table 1). Quantitative analysis of total phenolic and total flavonoid content in extracts of *Rosa damascena* are shown in table. 2 Plant phenolics constitute one of the major groups of compounds acting as a primary antioxidant or free radical terminators. Phenolic compounds such as flavonoids, phenolic acids and tannins are considered the major contributors to the antioxidant activity of vegetables, fruits or medicinal plants. The antioxidant activity of the phenolic compounds was attributed to its redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and have also metal chelating properties. The total phenolic and total flavonoid contents for the extracts of *Rosa damascena* flower petals are presented in table 2.

N=3, Values are expressed as Mean  $\pm$  SEM

Methanol extract contains high total phenolic and flavonoid contents equivalent to  $69.17 \pm 0.167$  mg/g GAE and  $76.28 \pm 0.29$  mg/g RE; whereas the aqueous extract

showed the lower content equivalent ( $49.01 \pm 0.167$  mg/g GAE and  $55.09 \pm 0.31$  mg/g RE respectively).

### Antioxidant Activity

#### DPPH Free Radical Scavenging Activity

DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517nm, which is induced by antioxidants. As shown in figure.1 *R. damascena* strongly scavenged the DPPH radical in a dose- dependent manner. It has been shown that the scavenging effects on the DPPH radical increases sharply with the increasing concentration of the extracts and standards to a certain extent and hence are said to be strongly dependent on the extract concentration. The IC<sub>50</sub> values of ascorbic acid, aqueous and methanol extract are shown in table 2.

#### Nitric oxide radical scavenging activity

Nitric oxide radical generated from sodium nitroprusside (SNP) at physiological pH was found to be inhibited by *R. damascena*. The scavenging of NO radical by the extracts was increased in dose dependent manner. Figure.2 illustrates a significant decrease in the NO radical due to the scavenging ability of extracts and ascorbic acid. Suppression of released NO may be partially attributed to direct NO scavenging, as the extracts of *R. damascena* decreased the amount of nitrite

generated from the decomposition of SNP *in vitro*. The IC<sub>50</sub> values of ascorbic acid, aqueous and methanol extract are shown in table 2.

#### Antimicrobial Activity

The antimicrobial potential of extracts was compared according to their zone of inhibition against the several pathogenic organisms. Antimicrobial screening of different extracts of *Rosa damascena* by ditch plate and cup plate method are given below. Both methanol and aqueous extracts of *Rosa damascena* were susceptible to all bacterial strains and fungal strains. Methanol extract showed highest susceptibility towards all strains of microorganisms. Aqueous extract and methanol extracts did not show any susceptibility towards *Aspergillus niger*. The presence of flavanoid and phenolic compounds in methanol extract of *Rosa damascena* may have attributed to its antibacterial activity. Both extracts at different concentrations were further analyzed by agar cup plate technique. The results obtained are given in table 4.

Methanol extract and aqueous extract showed good antibacterial activity at 40 mg and 80 mg respectively which was comparable with standard Chloramphenicol. However only methanol extract showed antifungal activity against *Candida albicans*. Aqueous extract did not showed antifungal activity.

The study showed that methanol extracts of *Rosa damascena* displayed a broad spectrum antimicrobial and antioxidant activities. The presence of flavonoids and phenolic compounds in the methanol extract may have partly contributed to the observed antioxidant and antimicrobial activities.

#### CONCLUSION

The results obtained represent a worthwhile expressive contribution to the antimicrobial and antioxidant activity of methanol extracts of *Rosa damascena*. Further studies are required for identification of active constituents responsible and to understand the mechanism of action of this plant.

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