



**PHYTOCHEMICAL ANALYSIS AND IN-VITRO ANTIOXIDANT AND  
ANTIBACTERIAL ACTIVITY OF AQUEOUS EXTRACTION FROM  
THE STEM OF *ROSA DAMASCENA MILL L.***

**DEEPIKA J<sup>1\*</sup>, DHARINISHREE S<sup>1</sup>, KIRUBHALINI S<sup>1</sup>, ARUN KUMAR J<sup>1</sup>,  
PRATHEEP M<sup>1</sup>, ARUN KUMAR R<sup>1</sup> AND ANANDHASELVAN S<sup>1</sup>**

**1:** Department of Pharmacy, Shri Indra Ganesan Institute of Medical Science, Manikandam,  
Tiruchirappalli, Tamil Nadu, India

**\*Corresponding Author: Dr. Deepika Jayaraman; E Mail: [deepikajavaraman238@gmail.com](mailto:deepikajavaraman238@gmail.com)**

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**ABSTRACT**

The present study evaluated the antioxidant and antibacterial activities and phytochemical investigation of aqueous extract of *Rosa Damascena Mill.* Stem. The antioxidant activity was evaluated by the DPPH assay method and antibacterial activity was determined by disc diffusion method. The aqueous extract showed high antioxidant activity (73.07%) and had antibacterial activity against both Gram-positive and Gram-negative bacteria. A higher inhibition zone was detected against *Staphylococcus aureus* -18mm, *Pseudomonas aeruginosa*-13mm, *Bacillus subtilis*-11mm, and *Escherichia coli*-9mm at 100µg/ml concentration. The minimum zone of inhibition was detected at 60µg/ml concentration. Preliminary phytochemical exploration indicated the presence of Alkaloids, Carbohydrates, Flavonoids, Saponins, Proteins, Starch, Free amino acids, Fixed oil and fat, and Steroids. The result indicated that the aqueous extract of *R.Damascena mill* stem possessed strong antibacterial and antioxidant properties and could be an important source of natural compounds for the development of a new drug.

**Keyword: Antioxidant, Antibacterial, Aqueous extract, Phytochemical, Rosa Damascena Mill.**

**INTRODUCTION**

*Rosa damascena mill L.*, commonly known as damask rose, is one of the most important

species of the *Rosaceae* family. *Rosaceae* are well-known ornamental plants and have been called the king of flowers [1]. *Rosa*

*damascena mill* is a hybrid between *R. gallica* and *R. Phoenicia* and is a member of the *Rosaceae* family with more than 200 species and 18000 cultivators around the world [2]. Plants are the richest source of antioxidant activity because they survive in an environment rich in harmful sun rays such as UV radiation [3]. *Rosa damascena* is a rich source of phytochemicals such as alkaloids, carbohydrates, flavonoids, terpenes, saponins, proteins, amino acids, etc.; *R. damascena* flowers also have a wide range of pharmacological effects [4]. *Rosa damascena* is a paramount medicinal drug and has several pharmacological and therapeutic actions such as antipyretic, antiseptic, antiemetic, anti-obstructive, analgesic, digestive, stomachic, liver tonic, cardiac tonic, brain tonic, general tonic, desiccant, detergent demulcent, palpitation, headache, constipation, mouth ulcer, etc., [4]. Bacteria are small unicellular organisms. The majority of bacteria are harmless, but some are detrimental. Examples: Gram-positive bacteria *Staphylococcus aureus*, *Bacillus subtilis* Gram-negative bacteria *Pseudomonas aeruginosa*, *E. coli*, etc. [5]. The medication that can treat bacteria are called antibacterial active agents. They are designed to inhibit or kill the infecting organism. Antioxidants can prevent or slow cell damage caused by free radicals, which are unstable molecules that the body produces as a reaction to the

environment and other pressures [6]. *Rosa damascena* could be a potential source of antioxidant and antibacterial activities. The present study investigated the antioxidant and antibacterial activity of *Rosa damascena* by in vitro methods.

## MATERIALS AND METHODS

### Collection of plant:

The investigation was carried out with *Rosa Damascena* in Shri Indra Ganesan College, Department of Pharmacy, Trichy. The stem of *Rosa damascena* was collected from Trichy, Tamilnadu, India. It was authenticated by Dr. L. John Peter Arulanatham, Director of Rapinat Herbarium, St. Joseph's College, Tiruchirappalli, Tamil Nadu.

### Preparation of aqueous extract:

The stem was washed thoroughly with tap water, cut into small pieces, shade-dried in the air at room temperature, and crushed to powder with an electric grinder. 20g of powder sample is added to 100mL and boiled for 5 minutes. The extract should be filtered by using Whatman's filter paper no 1 and air-dried at 28°C. The dried extract will be labelled and kept in air-tight bottles in the refrigerator at 4°C.

### Preliminary Phytochemical investigation:

It was carried out on an aqueous extract of *Rosa damascena*. A test for common phytochemicals was carried out by standard methods described in practical Pharmacognosy by Dr. C. K. Kokate and

Trease and Evans. The various chemical tests were carried out for the detection of chemical constituents viz., carbohydrates, alkaloids, Proteins, Phenolic compounds, tannins, saponins, glycosides, flavonoids, resins, fixed oils, starch, steroids, triterpenoids.

#### **ANTIOXIDANT ACTIVITY:**

##### **Determination of total flavonoid content (TFC)**

The total flavonoid content in plant extract was determined by the Aluminium chloride method. The reaction mixture consisted of 0.25mL of plant extract in 1.25mL distilled water then (NaNO<sub>3</sub>) was added and then the mixture was placed in the dark for 6 minutes then 0.15mL of 10% AlCl<sub>3</sub> was added and again incubated in the dark for 5 minutes. Now 0.5mL of NaOH and 0.275mL distilled water were added to the mixture. The set of standard solutions of Quercetin was prepared with concentration (20, 40, 60, 80, and 100µg/mL). The absorbance of the standard and the extract solution was measured against the reagent blank at 510nm with a UV/visible spectrophotometer. The total flavonoid content was determined from the calibration curve and expressed as milligrams of Quercetin equivalent (QE) per gram of extracts [7].

#### **IN VITRO ANTIOXIDANT ACTIVITY**

##### **DPPH assay method:**

The antioxidant activity of the sample was examined by stable DPPH free radical activity. Ethanolic solution of DPPH (0.05mM) (500µL) was added to 1000µL of aqueous extract with different concentrations (20-100µL). The freshly prepared DPPH solution was kept in the dark at 4°C. Then 96% (2.7 mL) of ethanol was added to the mixture and shaken vigorously. The mixture was kept to stand for 5 minutes at 540nm, absorbance was measured spectrophotometrically. Absorbance was set to zero by using ethanol. A blank sample containing the same amount of ethanol and DPPH was prepared. They all performed in triplicate. The radical activity of the tested samples, expressed as a percentage of inhibition was calculated.

$$\text{Percent (\% inhibition of DPPH activity)} \\ = [(A-B)/A] \times 100.$$

Where A and B - absorbance values of blank and sample, respectively. A curve of concentration versus percentage inhibition was plotted and the concentration required for 50% inhibition was determined [8, 9].

#### **ANTIBACTERIAL ACTIVITY OF SAMPLE**

##### **Collection of test pathogens:**

The antibacterial activity of the sample was exhibited against two gram-positive bacterial strains *Staphylococcus aureus* (MTCC 87), *Bacillus subtilis* (MTCC 441), and two gram-negative bacterial strains *Escherichia coli* (MTCC

443), *Pseudomonas aeruginosa* (MTCC 1688) were prepared as test organisms. All the bacterial strains were purchased from the Microbial Type Culture and Collection (MTCC) in Chandigarh, India.

#### **Determination of antibacterial activity by disc diffusion method:**

The samples of 60, 80, and 100 µg/mL were placed on the top of Mueller-Hilton agar plates. A filter disc diffusion method is used to evaluate the antibacterial

activity of the sample. Ten mL of Mueller-Hilton agar medium was poured into sterile Petri dishes (diameter 60 mm) and inoculated with the test organism. Sterile filter paper discs loaded with various concentrations of paper disc loaded with 5µg of amoxicillin was used as a positive control. The plates were incubated at 37°C for 24 hours the zone of inhibition was recorded in millimetres and the experiment was repeated twice.

## **RESULT AND DISCUSSION**

### **I. PHYTOCHEMICAL INVESTIGATION**

The Rosa Damascena stem is rich with alkaloids carbohydrates, flavonoids, saponins, protein & free amino acids fixed oil & fat, tannins& phenols, triterpenoids, and starch. The presence of phytochemicals is listed in **Table 1**.

### **II. Estimation of flavonoid content (TFC)**

The flavonoid content in the extract was expressed as µg quercetin equivalent. The aqueous extract of RD showed the highest amount of flavonoids. The standard calibration curve for total flavonoid content for standard Quercetin with aqueous extract is shown in **Graph 1**. The total flavonoid content for the aqueous extraction of *Rosa damascena* was found to be 76 mcg/mg (**Table 2**).

### **III. IN VITRO ANTIOXIDANT STUDY**

#### **Antioxidant activity of aqueous extract by DPPH assay method:**

Free radicals are known to play a definite role in a wide variety of pathological manifestations. Antioxidants fight free radicals and protect us from various diseases. They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defense mechanism. The result shows that the sample exhibits antioxidant activities at high concentrations when compared with standard ascorbic acid. The aqueous extract has 73.07% antioxidant activity at a concentration of 100µg/ml, while the ascorbic acid has 83.65% at the same concentration. Proton radical scavenging action is due to antioxidants measured by the DPPH radical scavenging assay (**Table 3**).

The extract showed a significantly higher inhibition percentage and positively correlated with the total flavonoid content of the extract. IC50 values obtained for the standard and sample were found to be 24.57 and 39.34µg/ml (**Graph 2, 3**). The stem of

this plant showed better antioxidant potential when compared to standard ascorbic acid by the DPPH method (**Chart 1**). It means that the aqueous extract of a plant at a higher concentration captured more free radicals formed by the DPPH assay method.

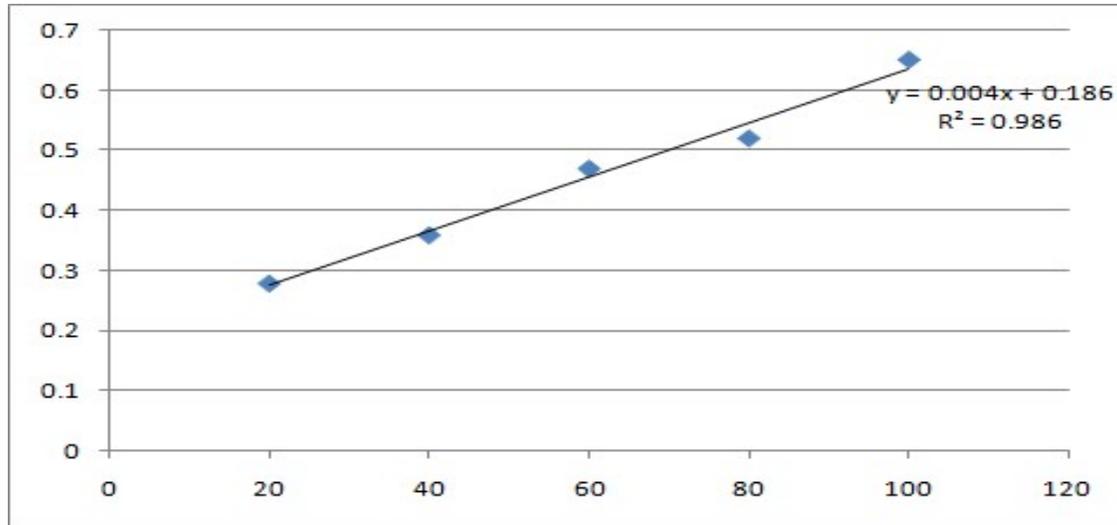
#### IV. ANTIBACTERIAL ACTIVITY OF SAMPLE

The extract was made with water (aqueous) and a qualitative test on various bacteria which included gram-positive and gram-negative bacteria by disc agar diffusion method. From the observation, the aqueous extract proves a significant zone of inhibition in all cultures. Hence the aqueous extract was performed the quantitative test at

various concentrations (60µg/ml, 80µg/ml, 100µg/ml) on gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) gram-negative (*Pseudomonas aeruginosa*, *Escherichia coli*). The result indicated that all the two types of bacteria have a maximum zone of inhibitory effect at 100µg/ml and a remarkable effect with other concentrations, while the last concentration 60µg/ml showed a minimum zone of inhibition effect. The antibacterial activity is shown in **Table 4**. Antibacterial activity was produced by the aqueous extract of *Rosa damascena* stem. Inhibited the growth of bacteria of both Gram-positive and Gram-negative are shown in **Figure 1-4**.

**Table 1: Data showing the presence of phytochemical constitution in aqueous stem extract of *Rosa Damascena***

S. No	PHYTOCONSTITUENTS	AQUEOUS EXTRACT
1.	Alkaloids	+
2.	Carbohydrate	+
3.	Glycoside	--
4.	Flavonoids	+
5.	Saponins	+
6.	Protein & Freeaminoacids	+
7.	Fixedoil & Fat	+
8.	Steroids	-
9.	Tannins & Phenol	+
10.	Triterpenoids	+
11.	Starch	+



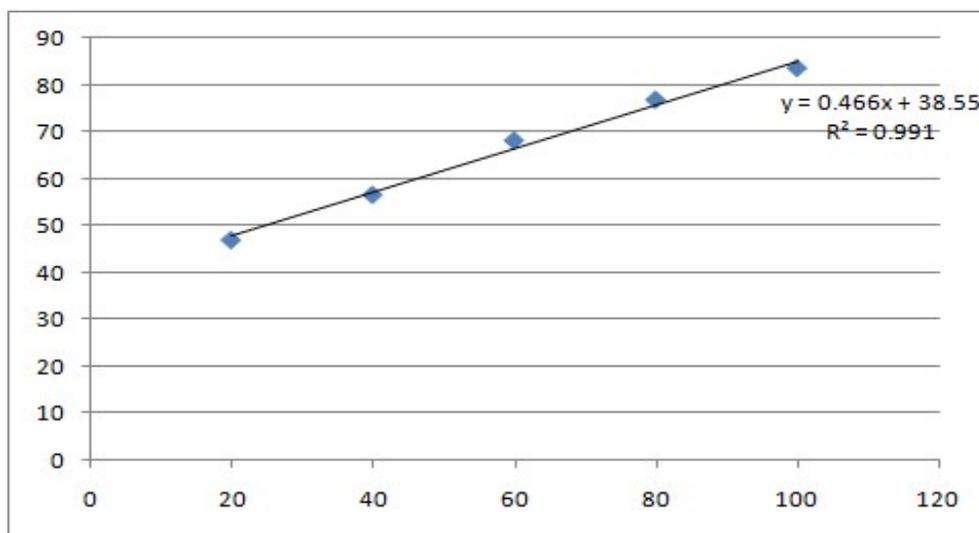
Graph 1: Standard calibration curve for total flavonoid content for standard Quercetin with aqueous extract  
 $Y=MX+C$ ;  $Y=0.004X+0.186$ ;  $X=Y-C/M$ ;  $X=0.49-0.186/0.004$ ;  $X=76\text{mcg/mg}$

Table 2: Total flavonoid content of aqueous extract of samples

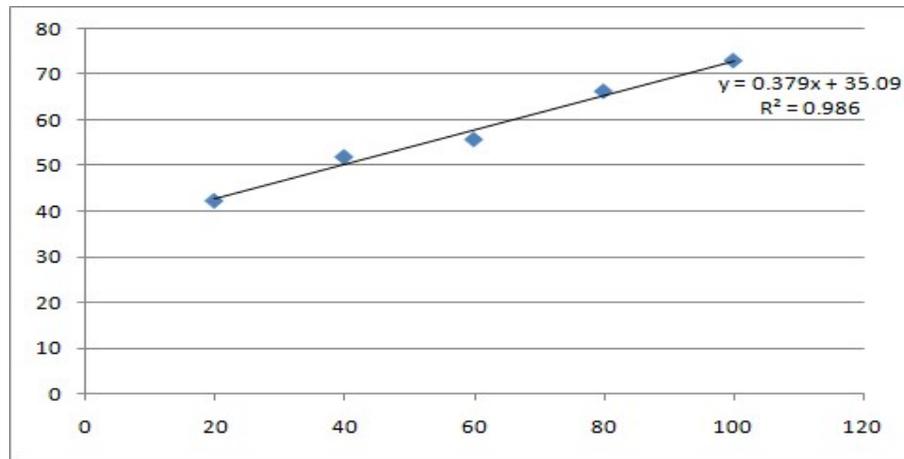
Total flavonoid contents in aqueous Sample	76mcg/mg
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Table 3: Antioxidant activity of aqueous extract by DPPH assay method

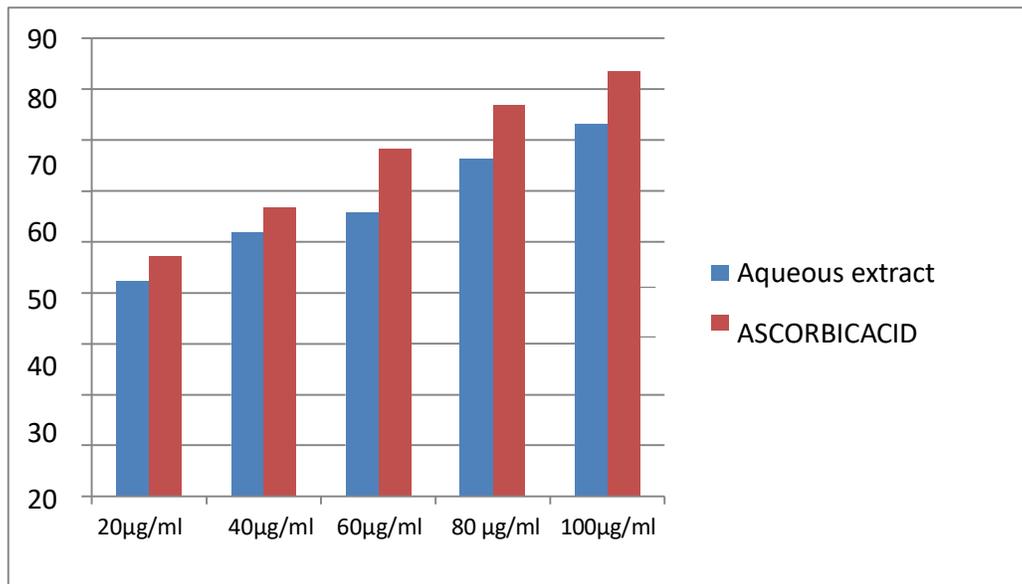
S. No.	CONCENTRATION ( $\mu\text{g/ml}$ )	ANTIOXIDANTACTIVITY DPPH%	
		ASCORBIC ACID	AQUEOUS EXTRACT
1.	20 $\mu\text{g/ml}$	47.11	42.30
2.	40 $\mu\text{g/ml}$	56.73	51.92
3.	60 $\mu\text{g/ml}$	68.26	55.76
4.	80 $\mu\text{g/ml}$	76.92	66.34
5.	100 $\mu\text{g/ml}$	83.65	73.07
	IC50value	24.57	39.34



Graph 2: IC50CALCULATION FORSTANDARDASCORBICACID  
 $Y=MX+C$ ;  $Y=0.466X+38.55$ ;  $X=Y-C/M$ ;  $X=5038.55/0.46$ ;  $X=24.57$



**Graph 3: IC50 CALCULATION FOR SAMPLE AQUEOUS EXTRACT**  
 $Y = MX + C$ ;  $Y = 0.379X + 35.09$ ;  $X = (Y - C) / M$ ;  $X = (50 - 35.09) / 0.379$ ;  $X = 39.34$



**Chart 1: Antioxidant activity of samples aqueous extract as compared to ascorbic acid by DPPH assay method**

**Table 4: In Vitro Antibacterial activity of the sample**

Samples	Concentrations (µg/ml)	Organisms/Zone of inhibition (mm)			
		<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>
Samples	60	4	7	7	5
	80	12	9	8	8
	100	18	13	10	9
Standard (Std) (Amoxicillin)	10 µl/disc	21	15	11	14
AQUEOUS	10 µl/disc	0	0	0	0

Figure: 1

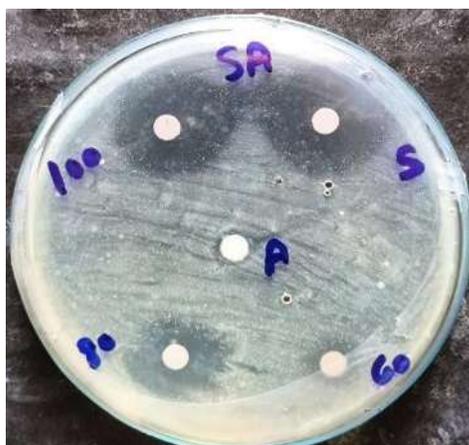
*Staphylococcus aureus*

Figure: 2

*Pseudomonas aeruginosa*

Figure: 3

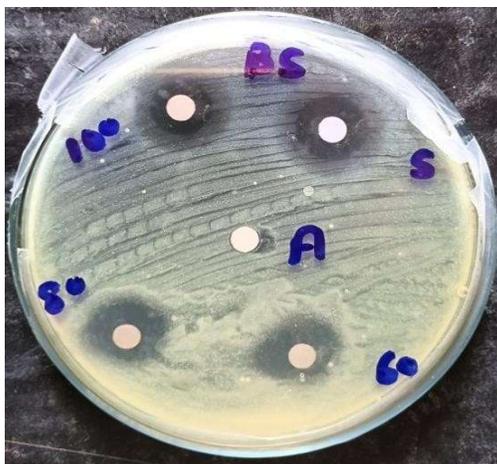
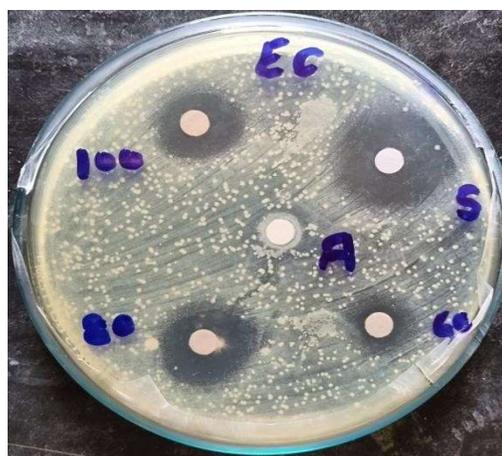
*Bacillus subtilis*

Figure: 4

*Escherichia coli*

## CONCLUSION

The aqueous extract of *Rosa Damascena* stem is a potential source of natural antioxidants and serves as an effective free radical. Hence, *Rosa Damascena* might be a good plant-based pharmaceutical product for several diseases caused by free radicals. From the result, it can be concluded that the *Rosa Damascena* stem of aqueous extract was

antibacterial in nature due to the presence of different types of active phytochemicals. Therefore, might be beneficial as antibacterial agents. The *Rosa Damascena* stem of aqueous stem of aqueous extract might be considered a safe supplementary therapy for long-term and effective management of antibacterial activity. Further phytochemical analysis of this plant will be helpful for the elucidation of

lead molecules. This *Rosa Damascena* is a useful medicinal plant and its further assessment is important which can provide help in the discovery of new antibiotics drug development in the market.

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