

**IN VITRO ANTIBACTERIAL ACTIVITY STUDY OF  
ROSA INDICA LINN. LEAVES EXTRACTS**

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**ABSTRACT**

To know the *in vitro* antibacterial activity of both the ethanolic and methanolic extracts of the leaves of both Pink Rose and Maroon Rose varieties of *Rosa indica* (Family-Rosaceae), present study was conducted. Both the extracts of each variety were used at 1mg/ml, 5mg/ml and 20mg/ml concentrations. While both the extracts of Pink Rose variety did not produce antibacterial activity at 1mg/ml, *Staphylococcus aureus* and *Escherichia coli* were sensitive to both the extracts at 5mg/ml and 20mg/ml in a dose-dependent manner. However, both ethanolic and methanolic extracts of Maroon Rose variety were only effective against *Staphylococcus aureus* at 20mg/ml. *Bacillus subtilis* was resistant to both the extracts of Pink Rose and Maroon Rose varieties. Moreover, like tetracycline (positive control) both the extracts of Pink Rose variety were more or less equally effective against *Escherichia coli* at 20 mg/ml.

**KEY WORDS-** Pink Rose, Maroon Rose, ethanolic extract, methanolic extract, antibacterial activity.

**INTRODUCTION**

Rose is a perennial plant of the genus *Rosa*, within the family Rosaceae. There are over hundred species of roses. They form a group of erect shrubs and climbing plants, with stems armed with sharp pickles. Flowers are large and showy and come out in many colours. Most species are native to Asia, Europe, North America and North West Africa. They are cultivated for their beauty and fragrance<sup>1</sup>.

Rose Tea (petals and leaves brewed as a tea) can bring down fever. It works as a diuretic to flush the toxins from the body. It can also relieve bronchial and chest congestion, provide relief from a sore throat and stop runny nose. Rose water has antiseptic properties and is used as an eye wash to treat eye irritation. Rose hips are used in cooking where they add flavour as well as nutrition. Rose oil is used for skin treatment to smooth and moisturise the skin and to relieve skin irritation<sup>2</sup>.

Till now reports on the antibacterial activity study using leaves of rose plants are scanty. However, Koday et. al. (2010) mentioned the antibacterial activity of rose petals<sup>3</sup>. Considering this information, we tried to explore the antibacterial activity of the leaves of Pink Rose and Maroon Rose varieties of Rose plant (*Rosa indica*).

**MATERIALS AND METHODS**

**Plant material-** The authenticated leaves of the plant *Rosa indica* (Pink Rose and Maroon Rose varieties) were collected from Chhend, Rourkela, during November

2010. The shade dried leaves were powdered and stored in a dessicator until evaporation.

**Preparation of extract-** The powdered leaves were passed through a sieve (No.40) and stored in a dessicator. For the extraction of the leaves, we followed the maceration method.

The powdered leaves (4 gm) were macerated in 30 ml of 95% ethanol for 3 days at room temperature. The resulting extract was filtered through a filter paper (Whatman No.1). The residue was further extracted using the same procedure. The filtrates obtained were combined and then evaporated to dryness. We followed the same method of extraction using methanol instead of ethanol<sup>4</sup>. After drying, both the extracts were dissolved in dimethylsulfoxide (DMSO)<sup>5</sup>.

**Agar well diffusion method-** In order to determine the antibacterial activity of the ethanolic and methanolic extracts of the leaves of both Maroon Rose and Pink Rose varieties of *Rosa indica*, the nutrient agar well diffusion method as described by Schillenger and Luke (1989) was performed. Sterile nutrient agar medium was inoculated with 0.1ml of fresh overnight nutrient broth culture of each bacterium (approx.10<sup>7</sup>CFU/ml) and poured into sterile petriplates<sup>6</sup>. For our study, we used bacterial suspensions of *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*. In each plate, wells of 6mm in diameter were punched using a sterile borer and the plates were allowed to dry for 5min<sup>6,7</sup>. For the study both ethanolic and methanolic extracts of each variety of the

plant were used at 1mg/ml, 5mg/ml and 20mg/ml concentrations<sup>6</sup>. Each concentration of the ethanolic and methanolic extracts of Pink Rose variety was, at first, dispensed separately into different wells using sterile micropipettes. Then both the extracts of Maroon Rose variety were used in a similar manner. In addition, DMSO (negative control), and tetracycline at a concentration of 25µg/ml (positive control) were also dispensed separately into different wells<sup>8</sup>. The volume of different solutions used in each well was 50 µl. After holding the plates at room temperature for 2 hours to allow diffusion of the extracts and controls into the nutrient agar medium, the plates were incubated at 37 °C for 24hrs. After the incubation period, the plates were examined for inhibition of the bacterial growth around the wells. The diameters of the zones of inhibition in each case were measured<sup>6</sup>.

## RESULTS

Both the ethanolic and methanolic extracts of Pink Rose variety did not produce any antibacterial activity at 1mg/ml. While those extracts at 5mg /ml showed moderate zones of inhibition against *Staphylococcus aureus* and *Escherichia coli*, maximum zones of inhibition were produced against them at 20mg/ml. *Bacillus subtilis* was resistant to both the ethanolic and methanolic extracts (Table-1).

Both ethanolic and methanolic extracts of Maroon Rose variety were only effective against *Staphylococcus aureus* at 20mg/ml. *Bacillus subtilis* and *Escherichia coli* were totally resistant to both the extracts of this variety (Table-2).

While tetracycline produced maximum zone of inhibition against *Staphylococcus aureus*, it was minimum in case of *Escherichia coli*. DMSO did not produce any zone of inhibition against the bacteria used in the study.

## DISCUSSION

It has been mentioned in the literature that rose petal is found to have antibacterial activity<sup>3</sup>. But antibacterial activity of the leaves of the rose plant has not been studied thoroughly. From our study, it may be mentioned that the Pink Rose variety possesses antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*.

Moreover, like tetracycline both the extracts of Pink Rose variety are more or less equally effective against *Escherichia coli* at 20 mg/ml. So, both the extracts of Pink rose variety are probably effective against Gram negative bacilli. On the other hand, the antibacterial efficacy of the Maroon Rose variety is not remarkable.

The leaves of the plant with different flower colours (included in the present study), have displayed variable antibacterial activities most probably due to their differences in the biochemical and phytochemical compositions. Considering all the findings, antibacterial activity study in detail using several extracts of the leaves of rose plant, particularly of the Pink Rose variety, may be performed to extend our present work. Then only the plant could be considered as a potential source of antibacterial agent.

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Table-1: Antibacterial activity pattern of the extracts and controls of the Pink Rose variety

Microorganisms	Zone of inhibition after 24 hrs(in mm)							
	Ethanolic Extract			Methanolic Extract			Tetracycline 25µg/ml	DMSO
	1mg/ml	5mg/ml	20mg/ml	1mg/ml	5mg/ml	20mg/ml		
<i>Staphylococcus aureus</i>	0	19.5	23	0	18	23	31	0
<i>Bacillus subtilis</i>	0	0	0	0	0	0	26	0
<i>Escherichia coli</i>	0	12	15.3	0	14	14.5	15	0

Table-2: Antibacterial activity pattern of the extracts and controls of the Maroon Rose variety

Microorganisms	Zone of inhibition after 24 hrs(in mm)							
	Ethanollic Extract			Methanolic Extract			Tetracycline 25µg/ml	DMSO
	1mg/ml	5mg/ml	20mg/ml	1mg/ml	5mg/ml	20mg/ml		
<i>Staphylococcus aureus</i>	0	0	22	0	0	21	32	0
<i>Bacillus subtilis</i>	0	0	0	0	0	0	26	0
<i>Escherichia coli</i>	0	0	0	0	0	0	19	0

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