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A Study on Antibacterial Properties of *Rosa indica* against Various Pathogens

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ABSTRACT

In the present study Antibacterial properties of Rosa indica was explored against different pathogens. Antimicrobial components were extracted by solvent extraction wherein methanolic and ethanolic extracts were prepared. The antibacterial nature of extracts was assessed by agar well diffusion method. The methanolic extracts of Red rose petals were found to be most effective against all the pathogens used, they gave a zone of inhibitions of 27 mm against Escherichia coli, 26mm against Pseudomonas aeruginosa, and 25 mm against Staphylococcus aureus which was far better than the zone of inhibition given by the standard antibiotic Tetracycline used throughout the study. The MIC(minimum inhibitiory concentration) for the methenolic as well as ethanolic extracts were determined and it was found to be ranging between 0.12 µg/ml to 0.65 µg/ml.

Key Words: Antibacterial properties, Agar well diffusion method, Zone of inhibition, medicinal plants.

INTRODUCTION

An anti-microbial is a substance that kills or inhibits the growth of microorganisms, such as bacteria, fungi, or protozoan. Antimicrobial drugs either kill microbes (microbiocidal) or prevent the growth of microbes (microbiostatic). Disinfectants are antimicrobial substances used on non-living objects or outside the body.

The history of antimicrobials begins with the observations of Pasteur and Joubert, who discovered that one type of bacteria could prevent the growth of another.

Before penicillin became a viable medical treatment in the early 1940s, no true cure for gonorrhea, strep throat, or pneumonia existed. Patients with infected wounds often had to have a wounded limb removed, or face death from infection. Now, most of these infections can be cured easily with a short course of antimicrobials.

Why herbal antimicrobials?

The use of Herbal antimicrobials is because of fewer side effects. Natural antimicrobial compound in plants have been found to possess antimicrobial activity. In addition, the antimicrobial property of medicinal plant may differ depending on the forms of added plants such as fresh dried or extracted forms. Searches for substance with antimicrobial activity are frequent and medicinal plant have been considered interesting by some researchers since they are frequently used in popular medicine as remedies of many infectious diseases . Medicinal plants have also been considered as healthy source of life for all the people.

For the long period of time, plants have been a valuable resource of natural products for maintaining human health. India has rich tradition in use of medicinal plants to develop drugs. According to world health organization any plant which contains substance that can be used for therapeutic purposes or which are precursors of chemo pharmaceutical semi synthetic new drug is reoffered as medicinal plant [Hassanali, 2003]. Medicinal plant would be the important source of obtaining a variety of drugs as phytochemicals are more specific, biodegradable and are supposed to have fewer side effects photochemicals offer unique platform for structural diversity and biological functionality which is indispensable for drug discovery [Verpoorte, 2002].

Nature is a source of Medicinal agent and an impressive number of modern drug have been isolated from natural sources, many based on there use in Traditional medicine. Various medicinal plants have been used for years in daily life to treat disease all over the world [Nair R, *et al.,* 2005]. Historically plant have been reported to be inspirational novel drug components, as plant derieved medecines have made large contributions to human health and well being [Astal ZY, *et al.*, 2005]. Natural products from plants offer new agents for antimicrobial use. A special feature of higher plants is there capacity to produce a large number of organic chemicals of high structural diversity the so called secondary metabolites [Naqri SAH, *et al.*, 1991].

Looking at the importance of herbal antimicrobials over others, and the ongoing research some of them being Antimicrobial and Antioxidant Properties of Secondary Metabolites from White Rose Flower [Joo SS, *et al.*,2010]*;* Antimicrobial Activity of Rose petals Extract Against Some Pathogenic Bacteria [Hirulkar NB, *et al.,* 2010]*;* Rose Petal Tea as an Antioxidant rich Beverage: Cultivar Effects [Vinokur Y, *et al.,* 2006]; Bactericidal activities of different Medicinal plants extracts against Ocular pathogen viz Corynebacterium McGinley [Koday NK, *et al.*, 2010]; Antibacterial activity of traditional medicinal plants used by Haudenosaunee peoples of New York State[Frank MF, *et al.,* 2010]; Cytokinin Activity in Rose Petals and Its Relation to Senescence. *Plant Physiol* [Mayak S, *et al.,* 1969] the present study was designed to explore the antimicrobial properties of a common plant Rose of the family *Rosaceae*.

MATERIALS AND METHODS

SAMPLES:

- **Plant Samples:**

Two different varieties of Rose plants (Red & Yellow flower varieties) were collected from HARSH NURSRY, Hazratganj, Lucknow.

- **Pathogens :**

Three different pathogens namely *Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa* available at MRD Life Sciences (P) Ltd. Lucknow, collected from IMTECH, Chandigarh, were subcultured and used throughout the project work.

PREPARATION OF PLANT EXTRACTS:

Different parts of the plant were used for preparation of the methanolic and ethanolic extract. 2 gm of powdered samples were dissolved in 20 ml of 70% ethanol and 80% methanol. Flasks were kept in dark for 2-4 days. The solutions were filtered and left in oven at 50ºC till the extract dried. The amounts of evaporated metabolites from methanolic and ethanolic extracts were dissolved in double amount of 100 mM Tris HCl buffer pH 8. Giving the concentration of antimicrobial extracts to be 500 µg/ml. 8 different samples were prepared and the list is shown in Table 1.

Table 1: Different Samples with Methanolic and Ethanolic Extracts.

Sample 1: Red Rose Petals	Methanolic (sample 1M)
	Ethanolic (Sample 1E)
Sample 2: Yellow rose Petals	Methanolic (sample 2M)
	Ethanolic (Sample 2E)
Sample 3: Red rose Leaf	Methanolic (sample 3M)
	Ethanolic (Sample 3E)
Sample 4: Yellow rose Leaf	Methanolic (sample 4M)
	Ethanolic (Sample 4E)
Sample 5: Red rose Stem	Methanolic (sample 5M)
	Ethanolic (Sample 5E)
Sample 6: Yellow rose Stem	Methanolic (sample 5M)
	Ethanolic (Sample 6E)
Sample 7: Red rose Root	Methanolic (sample 7M)
	Ethanolic (Sample 7E)
Sample 8: Yellow rose Root	Methanolic (sample 8M)
	Ethanolic (Sample 8E)

Antibiogram analysis:

NA media was prepared by dissolving all its components in distilled water, media along with petriplates was autoclaved. Media was poured into sterile petriplates under sterile conditions and left for solidification. After solidification 50 µl pathogens were spread on the different plates and wells were bored. 50 µl antimicrobial Samples and tetracycline were loaded in the wells. Plates were incubated at 37 ºC for 24 hours, and observed for zone of inhibition. The zone of inhibition by the sample was compared with Tetracycline which is used as a standard antibiotic.

Determination of minimum inhibitory concentration (MIC)

There were 12 Test tubes were taken and 3ml of NB was filled in each test tube and autoclaved. The different tubes were left to cool down to room temperature. After these, 500 µl of plant extract was poured using micropipettes into 2 test tubes. 2 set each containing 6 test tubes were made and the antimicrobial extract was serially diluted. The inoculated set was kept for incubation for 24hrs whereas the blank was preserved. Optical Density was read at 600nm for each inoculated test tube using the uninoculated test tube as blank.

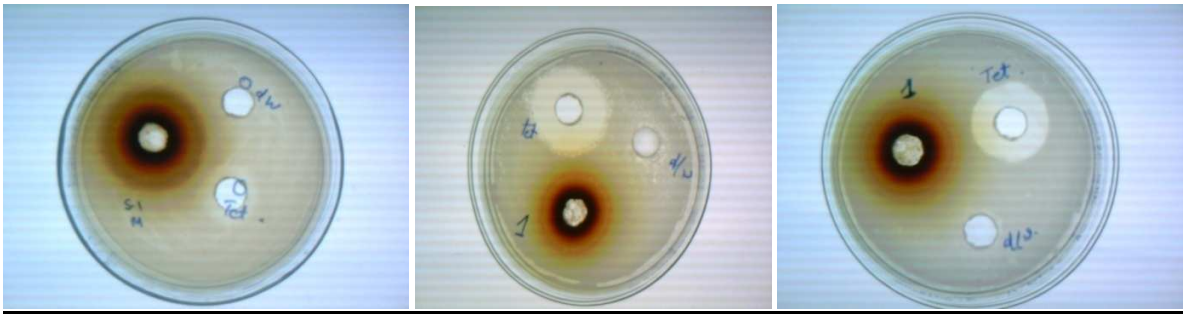
RESULTS AND DISCUSSION

Antibiogram analysis:

In order to check the antimicrobial activity of extracted plant samples, agar well diffusion method of Kirby bauer was used. With the help of this test we can determine that whatever culture we are using that is having antibacterial property or not. Table 1 below shows the results of zone of inhibitions observed for the antimicrobial extracts and the standard antibiotic tetracycline used throughout the study. It can be seen that the zones of inhibitions of the extracts 1M, 1E, 2M, 2E, 3M, 3E, 4M and 4 E are much better than the standard antibiotic Tetracycline. Figure 1-8 below show the photographs of the antibiogram performed against available pathogens.

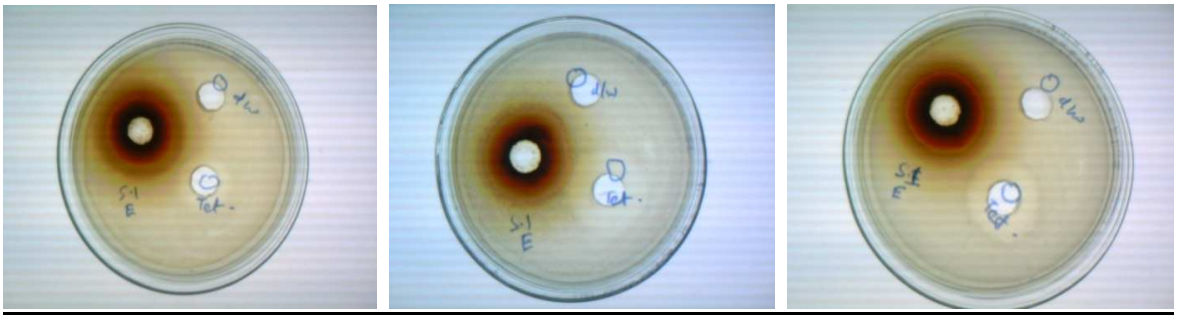
Table 2: Antibiogram of different Samples against different Pathogen.

Zone of Inhibition in mm			
Samples	***Staphylococcus aureus* / Tetracycline**	***Pseudomonas aeruginosa* / Tetracycline**	***Escherichia coli* / Tetracycline**
Sample 1M	**25/20**	**26/26**	**27/17**
Sample 1E	**26/20**	**25/26**	**27/17**
Sample 2M	**24/20**	**27/26**	**24/17**
Sample 2E	**24/20**	**24/26**	**25/17**
Sample 3M	**23/20**	**24/26**	**24/17**
Sample 3E	**24/20**	**21/26**	**25/17**
Sample 4M	**20/20**	**20/26**	**20/17**
Sample 4E	**21/20**	**19/26**	**18/17**
Sample 5M	**16/20**	**18/26**	**19/17**
Sample 5E	**17/20**	**20/26**	**19/17**
Sample 6M	**16/20**	**12/26**	**16/17**
Sample 6E	**11/20**	**12/26**	**13/17**
Sample 7M	**15/20**	**17/26**	**17/17**
Sample 7E	**17/20**	**18/26**	**15/17**
Sample 8M	**17/20**	**19/26**	**16/17**
Sample 8E	**17/20**	**18/26**	**19/17**



Staphylococcus aureus ***Pseudomonas aeruginosa*** ***Escherichia Coli***

Figure 1: Antibiogram of Methanolic Extracts of Red Rose Petals against Different Pathogen.

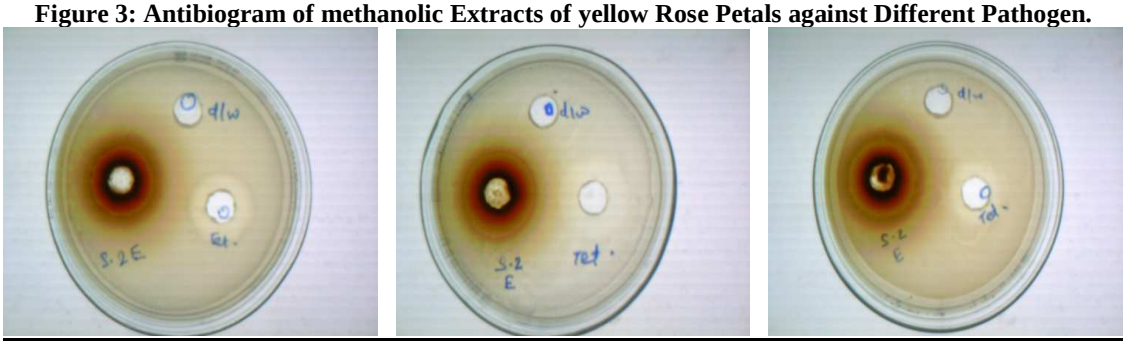


Staphylococcus aureus ***Pseudomonas aeruginosa*** ***Escherichia Coli***

Figure 2: Antibiogram of ethanolic Extracts of Red Rose Petals against Different Pathogen.

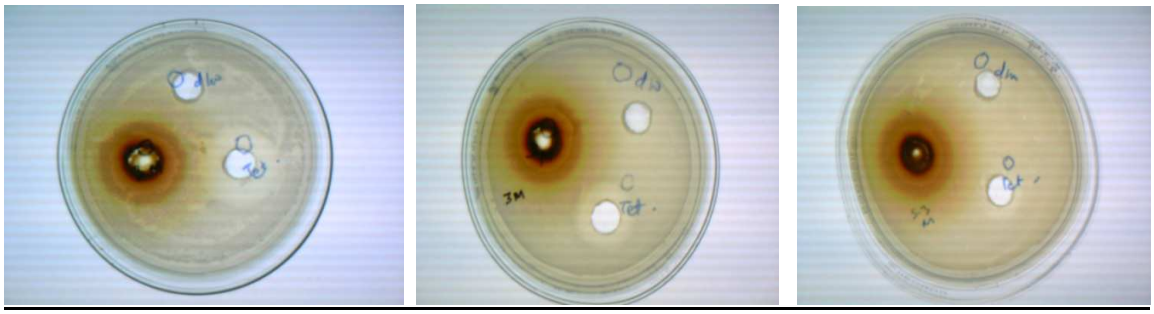
Staphylococcus aureus ***Pseudomonas aeruginosa*** ***Escherichia Coli***

Figure 3: Antibiogram of methanolic Extracts of yellow Rose Petals against Different Pathogen.



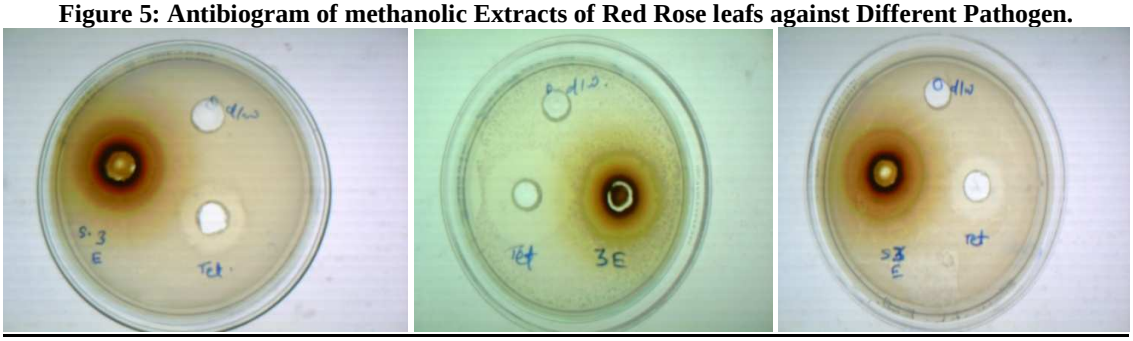
Staphylococcus aureus ***Pseudomonas aeruginosa*** ***Escherichia Coli***

Figure 4: Antibiogram of ethanolic Extracts of yellow Rose Petals against Different Pathogen.



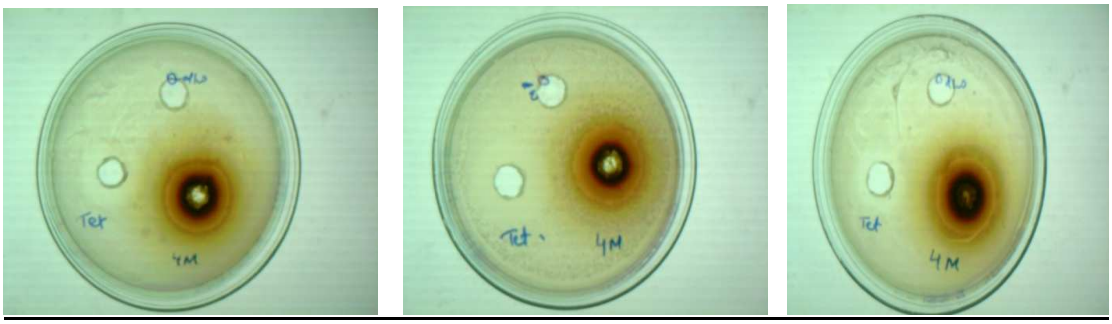
Staphylococcus aureus ***Pseudomonas aeruginosa*** ***Escherichia Coli***

Figure 5: Antibiogram of methanolic Extracts of Red Rose leafs against Different Pathogen.



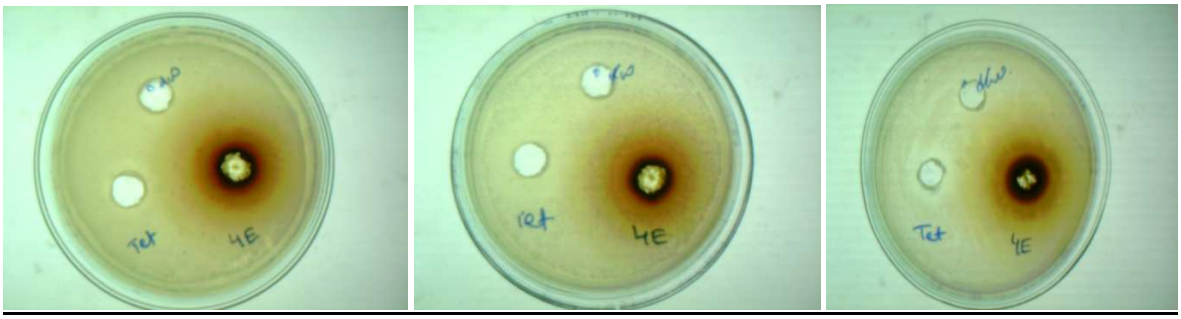
Staphylococcus aureus ***Pseudomonas aeruginosa*** ***Escherichia Coli***

Figure 6: Antibiogram of ethanolic Extracts of Red Rose leafs against Different Pathogen.



Staphylococcus aureus ***Pseudomonas aeruginosa*** ***Escherichia Coli***

Figure 7: Antibiogram of methanolic Extracts of yellow Rose leafs against Different Pathogen.



Staphylococcus aureus ***Pseudomonas aeruginosa*** ***Escherichia Coli***

Figure 8: Antibiogram of ethanolic Extracts of yellow Rose leafs against Different Pathogen.

Minimum inhibitory concentration (MIC):

The MIC for sample 1M, 1E, 2M, 2E, 3M and 3E was determined against *Escherichia coli* ,because it gave the best zone of inhibition in antibiogram analysis. 2M was determined against *Pseudomonas aeruginosa*. Table 2 below shows the MIC of the extracts against the respective pathogens against which maximum zones of inhibition were observed. The MIC of the methanolic extract of Red Rose Petals was determined against *Escherichia Coli*. It was seen that MIC is **40.81 µ**g/ml and The MIC of the ethanolic extract of Red Rose Petals was determined against *Escherichia Coli* and MIC was found to be **40.81 µ**g/ml.

Table 3: Minimal inhibitory concentration (MIC).

MIC (µg/mL)						
Pathogens	**Sample 1M**	**Sample 1E**	**Sample 2M**	**Sample 2E**	**Sample 3M**	**Sample 3E**
Escherichia coli	40.81	40.81	_	40.81	142.85	142.85
Pseudomonas aeruginosa	_	_	40.81	_	_	_

DISCUSSION

Herbal medicines are valuable and readily available resources for primary health care system. Undoubtedly the plant kingdom still hold many species of the plant containing substances of medicinal value that are yet to be discovered, though large number of plant are constantly being screened for this antimicrobial properties .This plants may prove to be rich source of compounds with possible antimicrobial properties. But more pharmacological investigation is necessary.

Plant extracts were prepared from dried samples in this research work as has been reported earlier by [Mahesh B, *et al*, 2008]. Both the ethanolic and methanolic extracts were taken for the antibiogram analysis in the present research work for the study of antimicrobial properties of *Rosa Indica*, which has been reported earlier by [Karthy ES, 2009].

Agar well diffusion method was used here in order to get the antibacterial properties of the different extracts against the test organisms, agar well diffusion has been used earlier by [Hirulkar NB, *et al .*, 2010], for antibacterial assay.

Methanolic extracts of Red rose petals gave the zone of inhibition of 25 mm, 26 mm, 27 mm against *staphylococcus aureus*, *pseudomonas aeruginosa* and *Escherichia coli* respectively. The ethanolic extract of red rose petals shows the zone of inhibition of 26mm, 25mm, 27mm against *Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli* respectively. Earlier also zone of inhibitions of 30mm, 32mm, 21mm against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* have been reported by [Hirulkar NB, *et al*., 2010].

The Minimum Inhibitory concentration (MIC) was also performed in order to know the minimum inhibitory concentration of the extract by broth dilution method and the MIC was found to be ranging between 0.12-.65µg/ml earlier also MIC of rose extract has been reported to between 5.0-18.0 mg/ml by [Vieira GDV, 2009].

CONCLUSION

The result of the present investigation shows the antibacterial nature of extracts of different parts i.e., (petals, leafs, stem and root) of *Rosa Indica*. Each of the plant parts was extracted in its dry powder form using ethanol and methanol. The data obtained demonstrated that the antimicrobial activity depends on the plant parts, solvent used for extraction and the test organism tested for susceptibility assay.

Methenolic extracts of Red Rose petals were found to be most effective against all the pathogens, followed by the ethanolic extracts of the same. Other plant parts also had antibacterial properties. So it can be concluded that Methenolic extraction procedure is the best in order to get antibacterial components from Rose plants.

The efficacies of all the extracts were approximately more than that of the antibiotic, Tetracycline. Tetracycline shows average zone of inhibition of 23mm against all the available test organism.

The MIC value of Samples 1M,1E,2M,2E,3M,3E which shows the best zone in antibiogram analysis can was tested against the pathogens MIC for the ethanolic as well as methanolic extract ranges from **0.12 µg/ml - 0.65 µg/ml** . It is apparent from the result that the MIC value are lower thus showing there efficacies, that is they are effective in very low concentration which is the need of the time, more activity is less concentration.

The future prospects of present research work includes isolation and purification of the therapeutic antimicrobials from the active extract and there further pharmacological evaluation by several method such as – NMR , MS , GC-M S , TLC,HPLC.

The purified antimicrobial can also be characterized for the effect of various cations in order to check there effects on the total effectiveness of the antimicrobial.

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