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THE STUDY ON ANTIMICROBIAL ACTIVITY OF *ROSA INDICA*

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ABSTRACT

In the present investigation, 14 bacterial strains found to be positive during primary screening (crowded plate technique) were subjected to secondary screening and isolate MJSS07 and MJSS14 showing maximum antimicrobial activity was selected for further studies. Strain improvement was performed by U.V.(ultra violet) and EtBr treatment.6µg/ml EtBr was found to be enhanced animicrobial activity and culture was maintained for further studies. The selected isolate was identified as *Bacillus subtilis* and *Bacillus megaterium* on the basis of bergey's manual. During the study of physiochemical factor of bacterial isolate MJSS07 & MJSS14 the maximum growth was observed at a temperature 37° C and a pH of 7.0.

Key words: Bacterial activity, Antibiogram analysis, Agar well diffusion, Solvent extraction

INTRODUCTION

Medicinal plants have always been considered a healthy source of life for all people. Therapeutically properties of medicinal plants are very useful in healing various diseases and the advantage of these medicinal plants is being 100% natural. Nowadays people are being bombarded with thousand of unhealthy products, the level of sensibility in front of diseases is very high and that's why the use of medicinal plants can represent the best solution. Natural antimicrobial compounds in plants were found to possess antimicrobial activity **[1,2].**

In addition, the antimicrobial property of medicinal plants may differ depending on the forms of added plants, such as fresh, dried, or extracted forms. Searches for substances with antimicrobial activity are frequent and medicinal plants have been considered interesting by some researchers since they are frequently used in popular medicine as remedies for many infectious diseases **[3]**.

The realization that many infectious pathogenic organisms are fast developing resistance(s) against the prevailing drugs has necessitated a search for new sources of antibacterial compounds. In the course of their life cycle, plants encounter infection by a variety of viruses, bacteria, fungi and parasites specific to them. They are expected to synthesize a variety of secondary metabolites capable of providing them protection against the infectious agent(s). Antibacterial activities have been detected in chemicals extracted from root, stem, leaves, flowers, fruits and seeds of diverse species of plants **[4,6].**.

Emerging and re-emerging infectious diseases and spread of deadly drug-resistant strains pose a challenge to public health care services. Several micro-organisms derived antibiotics are currently in use to treat a variety of infectious human disease **[5]**.

Most of them have a limited antimicrobial species of the pathogen, some even lead to serious side effects. Broad spectrum antibiotics lead to killing friendly bacteria in the colon, allowing yeast and other unfriendly microbes to multiply out of control. Some are synthetic compounds.

Therefore action must be taken to control the use of antibiotic, develop new drugs either synthetic or natural **[7,9]**.

Efforts are thus being directed to identify antibiotic sources other than traditional microorganisms **[10]**.

Rosa indica is a perennial shrub the leaves are alternate and pinnately compound, with sharply toothed oval-shaped leaflets. The plant's fleshy edible fruits, which ripens in the late summer through autumn, is called rose hip. Rose plants range in size from compact, miniature roses, to climbers that can reach 7 meters in height. Species from different parts of the world easily hybridize, which has given rise to the many types of garden roses **[11]**.

The leaves of most species are 5-15 centimetre long, pinnate, with (3) 5-9 (13) leaflets and basal stipules; the leaflets usually have a serrated margin and often a few small prickles on the underside of the stem. The remaining part after shedding of flowers is called "Rose hip", which have high antimicrobial activity (Yi, 2007).They have a wide spectrum of pharmacological properties including free-radical scavenging, antioxidant, anti-tumour and antibacterial activity **[12,13]**. Extracts from the leaves and hips of wild roses have been found useful for treatment of disease such as flu, colds and inflammatory states (Nowak, 2004). They also have antioxidant, antibacterial, antiviral and anticancer activity **[14]**.

MATERIALS AND METHODOLOGY

Collection of sample:

Fresh and healthy plant parts of *Rosa indica* were collected from plant nursery, lucknow, U.P., India. The plant materials were washed thoroughly with tap water followed by sterilized distilled water. They were dried in hot air oven at 50^0C and used as raw materials for the extraction of antimicrobial compounds **[15]**.

Preparation of plant extracts:

The plants raw materials were dipped into respective polar and nonpolar solvents.

Further these samples were filtered by using whatsmann filter paper and allowed to evaporate the solvents.**[11,12]**.

Screening of cultures for their antimicrobial activity:

The crude extracts were prepared and then the antibacterial activity was analysed against gram positive and gram negative strains. *Escheriachia coli, Pseudomonas aeruginosa, Staphylococcus aureus.* The tests were carried out by using agar well diffusion method **[16, 17].**

RESULTS

Antibiogram of Rosa indica:

1. Antimicrobial activity of R. indica (red rose) leaves extract against gram positive and gram negative microorganism.

Antibiogram against *P. aeruginosa* was maximum followed by *Bacillus sp.* and *E .coli* whereas least in *S.aureus*. Table 1& fig. 1, below show the zone of inhibition obtained in all the cases discussed.

Table 1 Antibiogram of red Rosa indica leaves extract against test microorganism

S.NO.	Test organism	Zone of inhibition (in mm)
1	*Bacillus sp.*	16.0
2	*Pseudomonas aeruginosa*	17.5
3	*Escherichia coli*	15.5
4	*Staphylococcus aureus*	15.0

2. Antibacterial activity of R. indica (yellow rose) leaves extract against gram positive and gram negative microorganism.

Antibiogram against *P. aeruginosa* was maximum followed by *Bacillus sp.* and *E. coli* whereas least in *S.aureus*. Table 2& fig. 2, below show the zone of inhibition obtained in all the cases discussed.

Table 2. Antibiogram of Yellow Rosa indica leaves extract against test microorganism

S.NO.	Test organism	Zone of inhibition (in mm)
1	*Bacillus sp.*	15.0
2	*Pseudomonas aeruginosa*	16.0
3	*Escherichia coli*	15.0
4	*Staphylococcus aureus*	14.5

3. **Antimicrobial activity of R. indica (pink rose) leaves extract against gram positive and gram negative microorganism.**

Antibiogram against *P. aeruginosa* was maximum followed by *S.aureus* and *E.coli* whereas least in *P. aeruginosa.* Table 3& fig. 3, below show the zone of inhibition obtained in all the cases discussed.

Table 3. Antibiogram of Pink Rosa indica leaves extract against test microorganism

S.NO.	Test organism	Zone of inhibition (in mm)
1	*Bacillus sp.*	16.5
2	*Pseudomonas aeruginosa*	21.0
3	*Escherichia coli*	17.5
4	*Staphylococcus aureus*	18.0

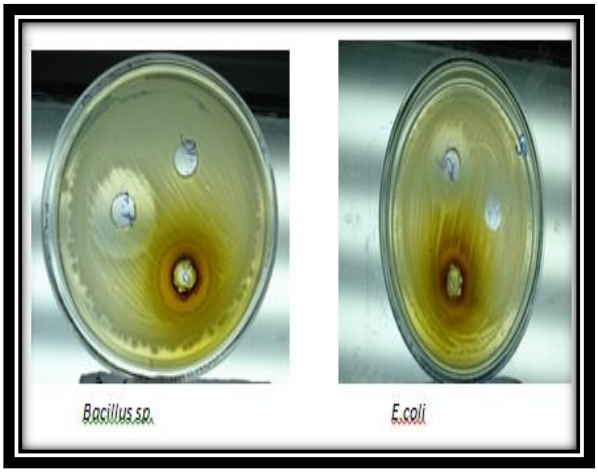

Figure – 1: Antibiogram of red R. indica leaves against gram positive and gram negative bacteria .

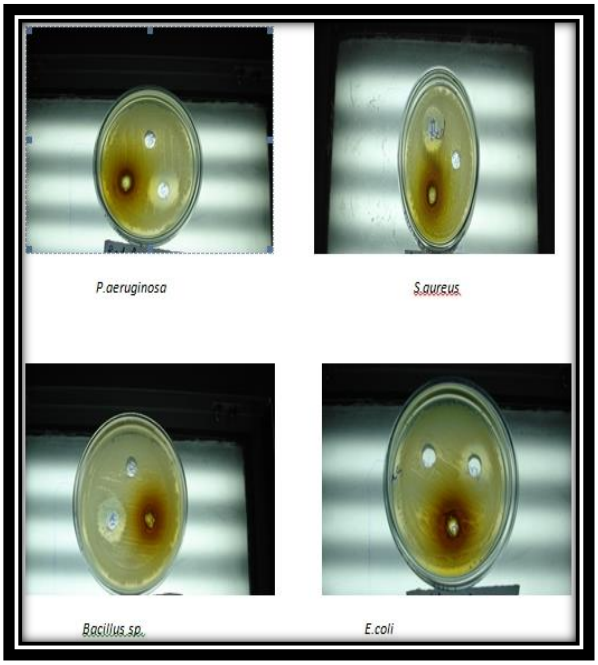

Figure – 2: Antibiogram of yellow R. indica leaves against gram positive and gram negative bacteria

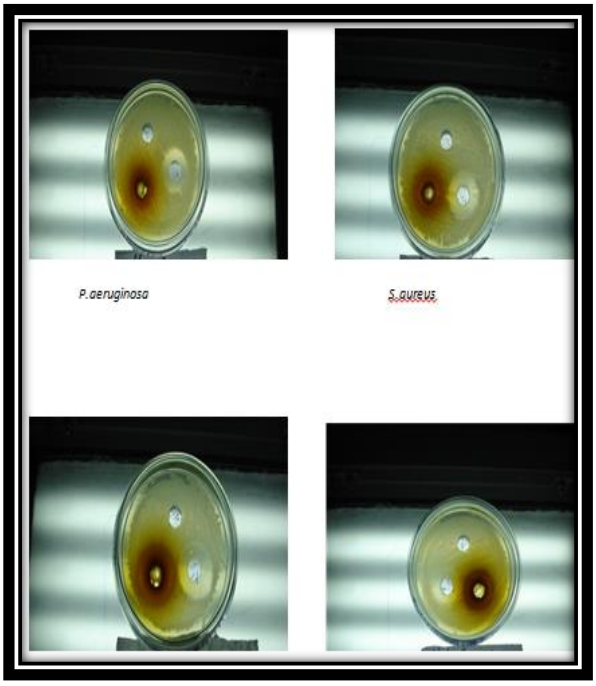

Figure – 3: Antibiogram of pink R. indica leaves against gram positive and gram negative bacteria .

Results for minimum inhibitory concentration

1.Red. R.indica Leaves sample:

Red R.indica leaves were subjected to get the MIC against *P. aeruginosa and it was found* to be 0.5gm/ml for the test of microorganism studied here below show the MIC obtained.

TEST TUBES	CONC. OF EXTRACT(in µg/ml)	O.D. AGAINST *P. aeruginosa*
BLANK	Same as 1, 2, 3, 4, 5, and 6 respectively	0.0
1	142.85	0.08
2	40.81	0.32
3	11.66	0.43
4	3.33	0.59
5	**0.95**	**0.44**
6	0.27	0.67

2. Yellow. R. indica Leaves sample

Yellow R.indica leaves were subjected to get the MIC against *P. aeruginosa and it was found* to be 0.5gm/ml for the test of microorganism studied here below show the MIC obtained.

TEST TUBES	CONC. OF EXTRACT(in µg/ml)	O.D. AGAINST *P.aeruginosa*
BLANK	Same as 1, 2, 3, 4, 5, and 6 respectively	0.0
1	142.85	0.00
2	40.81	0.08
3	11.66	0.20
4	3.33	0.51
5	**0.95**	**0.40**
6	0.27	0.54

3. Pink. R.indica Leaves sample:

Pink R.indica leaves were subjected to get the MIC against *P. aeruginosa* and it was found to be 0.5gm/ml for the test of microorganism studied here below show the MIC obtained.

TEST TUBES	CONC. OF EXTRACT(in µg/ml)	O.D. AGAINST *P. aeruginosa*
BLANK	Same as 1, 2, 3, 4, 5, and 6 respectively	0.0
1	142.85	0.00
2	**40.81**	**2.00**
3	11.66	0.50
4	3.33	0.52
5	0.95	0.41
6	0.27	0.39

Effects of magnesium ion:

Effects of magnesium ion on Antimicrobial activity of Pink Rose sample is the best result than Red and Yellow Rosa indica against *pseudomonas aeruginosa*.

Table 2. Effects of magnesium ion on Antibiogram of Yellow Rosa indica leaves extract against test microorganism.

S.NO.	Conc. Of Mg^{++}	Zone of inhibition (in mm}
1.	0%	16.0
2.	1%	16.5
3.	2%	18.5
4.	5%	19.0
5.	10%	19.5

2. **Extract sample of red Rose:**

Effects of magnesium ion on Antimicrobial activity of Red Rose sample are the best result than Yellow Rose wherever less effective than pink rose against *pseudomonas aeruginosa*.

S.NO.	Conc. Of Mg^{++}	Zone of inhibition (in mm}
1.	0%	18.0
2.	1%	16.0
3.	2%	13.5
4.	5%	12.0
5.	10%	11.0

3. **Extract sample of yellow Rose:**

Effects of magnesium ion on Antimicrobial activity of yellow Rose sample is less effective than pink and red rose against *pseudomonas aeruginosa.*

S.NO.	Conc. Of Mg^{++}	Zone of inhibition (in mm}
1.	0%	15.0
2.	1%	11.5
3.	2%	11.5
4.	5%	11.0
5.	10%	10.5

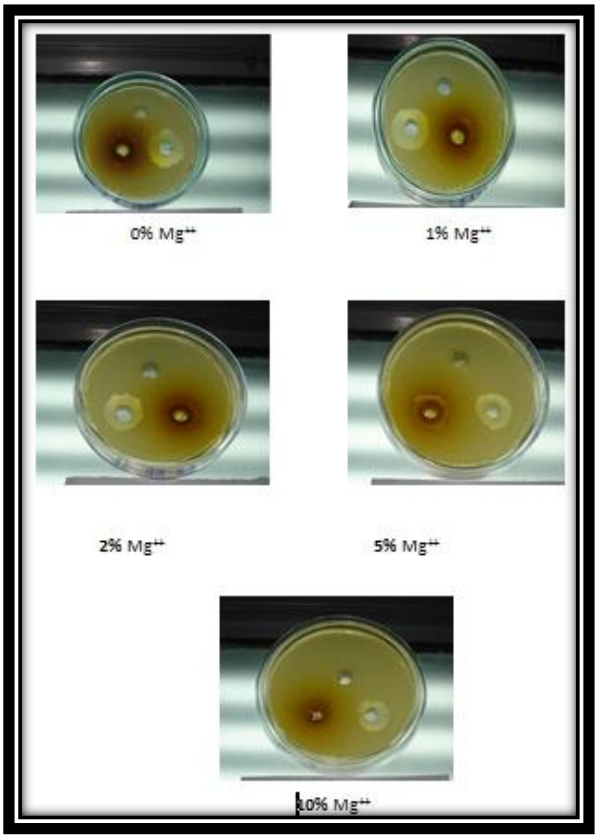

Figure – 4: Effect of Magnesium ion on Antibiogram of red R. indica leaves against *Pseudomonas aeruginosa.*

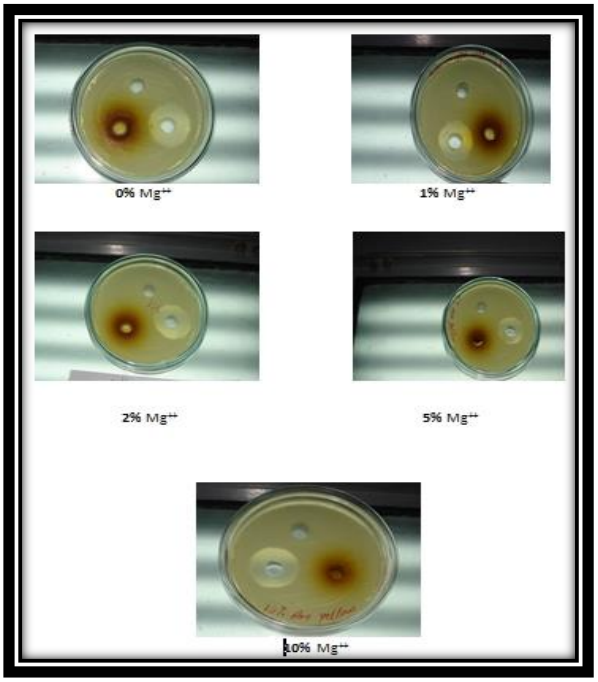

Figure – 5: Effect of Magnesium ion on Antibiogram of yellow R. indica leaves against *Pseudomonas aeruginosa.*

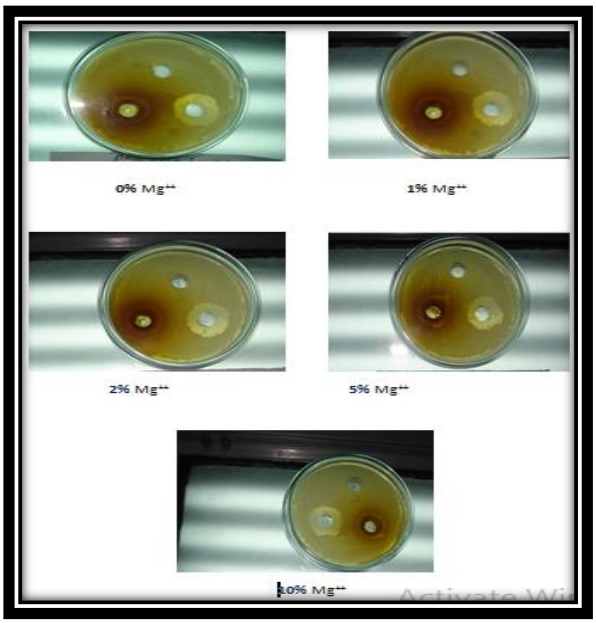

Figure – 6: Effect of Magnesium ion on Antibiogram of pink R. indica leaves against *Pseudomonas aeruginosa.*

DICUSSION

Herbal medicines are a valuable and readily available resource for primary health care and complementary health care systems. Undoubtedly, the plant kingdom still holds many species of plants containing substances of medicinal value that have yet to be discovered, though large numbers of plants are constantly being screened for their antimicrobial effects. These plants may prove to be a rich source of compounds with possible antimicrobial activities, but more pharmacological investigations are necessary.

Plant Extracts were prepared from dried samples in this research work as has been reported earlier by Robinson *et al.*, 2009**.** Methanolic extracts of various plant samples *R. indica* were taken for the antibiogram studies in the present research work, earlier also Nair *et al.*, 2004; Yi, *et al.*, 2007 and Bonev *et al.*, 2008, have reported that Methanolic extracts show best antimicrobial activities.

Agar well diffusion method was used here in order to get the antibiogram of R.indica Methanolic extracts against Test microorganisms as has been performed earlier by Bonev *et al.*, 2008. MIC was also done to know the minimum inhibitory concentration of the extract by broth dilution method as experimented earlier by Wikler *et al.,* 2009.

R. indica var. pink leaf gave a zone of inhibition of 16.5mm, 18.0mm, 17.5mm, and 21.0 mm against *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Bacillus* sp. respectively. Zone of inhibition ranging from 5.24 mm to 21.83 mm has been reported earlier by Robinson *et al.*, 2009.

R. indica var. red and *R .indica* var. yellow gave a zone of inhibition of 16.0mm, 15.0mm, 15.5mm, and 17.5 mm and 15.0mm, 14.5mm, 15.0mm, 16.0mm respectively against *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Bacillus* sp. respectively. Present work is one of the few studies being carried on Methanolic extract have been performed by Yi, O. *et al.*,

Effect of magnesium ion on R.indica var. Pink leaves gave the zone of inhibition of 16.0mm, 16.5mm, 18.5mm, 19.0mm, and 18.5mmto 0%, 1%, 2%, 5%, and 10% conc. of magnesium ion against Pseudomonas aeruginosa respectively.

Effect of magnesium ion on R.indica var. red leaves gave the zone of inhibition of 18.0mm, 16.0mm, 13.5mm, 12.0mm, and 11.0mm to 0%, 1%, 2%, 5%, and 10% conc. of magnesium ion against Pseudomonas aeruginosa respectively.

Effect of magnesium ion on R.indica var. yellow leaves gave the zone of inhibition of 15.0mm, 12.0mm, 11.5mm, 11.0mm, and 10.5mm to 0%, 1%, 2%, 5%, and 10% conc. of magnesium ion against Pseudomonas aeruginosa respectively.

CONCLUSION

Result of the present investigation reveal the antibacterial nature of extract of Rosa indica var. red, pink and yellow. All variety of Rosa indicia leaves was extracted in its dry powder form using methanol. Data obtained demonstrates that the antibacterial activity of plant depends upon the plant parts, extract concentration and the test micro organism tested for susceptibility assay.

Methanolic extract of Rosa indica leaves were found to be more susceptibility to gram positive bacteria as compaired to gram negative bacteria. This may be due to difference in their cell wall structure. Antibiogram of pink Rosa indica leaves against *Bacillus sp.* was maximum followed by *S. aureus* then *of E.coli* and *P.aeruginosa* Antibiogram of pink Rosa indica showed best result among the three varieties of Rosa indica.

Effect of Magnesium ion on Antibiogram of red Rosa indica leaves Methanolic extract against *Pseudomonas aeruginosa* was maximum zone of inhibition than yellow and pink Rosa indica.

Control experiment using sterile distilled water showed no zone of inhibition against any bacteria. The efficacies of all extract were less than that of the standard antibiotic as tetracycline. Tetracycline showed average zone of inhibition of 22.0mm against all test microorganism.

The future prospects of the present research work include isolation of the therapeutic antimicrobial from the Rosa indica leaves and carry out further purification and pharmacological evaluation by several method such as NMR,MS GC-MS,TLC, HPLC to study of the effect of different elicitors as heavy metal, Cations, anaions on antimicrobial property of plant extract and screening of several more RAPD markers in order to confirm the genetic diversity among several different varieties selected in our study. Showing different antimicrobial activity against same microorganism need further screening with some more unique primer using the same genomic DNA molecule.

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