

## Antibacterial activity of *Hibiscus rosa sinensis* and *Calendula officinalis* flowers extract against various Pathogen

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**Abstract** - From the long period of time, plants have been a valuable resource of natural products for human health. Some of plants leaves showing antimicrobial activity. The Primary benefit of using plant derived medicines is that they are readily affordable and accessible and showing fewer side effect. In the present study, I have evaluated that antibacterial activity of *Hibiscus rosa sinensis* flowers and *Calendula officinalis* flowers extract against some clinical isolates of bacteria. The crude preparation of *Hibiscus rosa-sinensis* flowers were tested against Gram Positive- *Staphylococcus aureus* and Gram negative- *Salmonella typhimurium*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*. Simultaneously Standard antibiotic solution of ampicillin is used. *Calendula officinalis* flowers crude extract antimicrobial activity was evaluated against Gram Positive- *Staphylococcus aureus*, *Bacillus subtilis*. Gram negative - *Pseudomonas aeruginosa*, *E.coli*, *Salmonella thyphi*. Gram negative bacterium - *Pseudomonas aeruginosa* showed maximum zone of inhibition against *Hibiscus rosa-sinensis* flower extract and *E.coli* against *Calendula officinalis* among these organisms. Simultaneously standard antibiotic solution of ampicillin was used. This experiment was done by agar gel diffusion method. The flower materials can be taken as an alternative source of antibacterial agent against the human pathogens. There may be possible use of these flowers extract for making drugs. Further pharmacological and clinical studies are required. These extract may be tested against multiple drug resistance microbes.

**Key words:-** *Hibiscus Rosa Sinensis*, *Calendula Offcinalis*, *Pseudomonas Aeruginosa*, *Salmonella Typhimurium*

### INTRODUCTION

There are very wide range of pathogenic bacteria so there are many variety of diseases caused by them. Medicinal plants have been using to cure many diseases from the ancient time. Plants are the best sources of natural products to maintain human health. Due to development of microbial resistance on antibiotics, researchers are trying to introduce antimicrobial activity of plants. Natural antimicrobial compounds in plants have been found to possess antimicrobial activity. Interest has been revive in herbal medicine due to fewer side effects and limited ability of synthetic pharmaceutical products to control major diseases. Herbal treatment is one possible way to treat disease caused by multidrugresistant bacteria.

*Hibiscus rosa-sinensis* is very popular in India. It is an evergreen shrub which is in leaf all the years. It's beneficial effects are also reported in ancient Indian Medicinal literature. (Nadkarni et.al 1976) *H.rosa sinensis* flowers are used in the treatment of excessive and painful menstruation

,venereal diseases, mumps, sores and coughs.(Duke and Ayensu,1985;chopraet.al,1986).Flower extracts of *H. rosa-sinensis* was tested against Gram Positive- *Staphylococcus aureus* and Gram Negative- *Salmonella typhimurium*, *E.coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*. *Calendula officinalis* also known as pot marigold. It is one of the herbal medicine. The flower of *Calendula officinalis* is used to make herbal drugs. It is a medicinal plant and also used in rash and itching treatment. *C. officinalis* flowers having antiseptic and antibacterial property. *C. officinalis* was tested against Gram Positive- *Staphylococcus aureus*, *Bacillus subtilis* and Gram Negative- *Pseudomonas aeruginos*, *E.coli*, *Salmonella thyphi*.

### MATERIALS AND METHODS

This study was done in Department of Microbiology, Shri Umiya Kanya Mahavidyalaya, Rau, Indore, India

#### Collection of flowers:-

Fresh flowers of *H.rosa-sinensis* and *Calendula officinalis*

were collected from college campus of Shri Umiya Kanya Mahavidyalaya at Indore.

#### Preparation of plants extracts:-

70 g *H.rosa-sinensis* and 50 g *Calendula officinalis* fresh flowers were taken and washed them 3-4 times with tap water, and then distilled water. The fresh leaves were cut, dried and grinded to produce paste. The paste material of *H.rosa-sinensis* was mix in ethanol to prepare ethanolic suspension . Ethanol is used because it is a good solvent to prepare extract of plant material. The paste material of *Calendula officinalis* was mix in petroleum ether to form extracts. It was found during the study that petroleum ether is a good solvent for *Calendula officinalis*. The collected extracts were concentrated by evaporation under room temperature. Material should be free from ethanol and petroleum ether. Solvent free materials were then mix with distilled water and then centrifuge at 5000 rpm for 15 minutes . Supernatant were used for experiment.

#### Preparation of standard antibiotic:

Simultaneously, standard antibiotic solution of ampicillin was prepared. Add 1.0 mg ampicillin in 1 ml distilled water.

#### Preparation of inoculums:-

Stock culture were maintained at low temperature. For this experiment active cultures were prepared by transferring loop full stock culture into nutrient broth. Placed nutrient broth tubes in incubator for 24 hrs.at 37°C.

#### Antimicrobial activity test:-

The antimicrobial activity of *H. rosa-sinensis* and *Calendula officinalis* were tested against some clinical isolates by agar well diffusion method. Nutrient broth and nutrient agar medium were prepared and sterilized in Autoclave. Each plate of nutrient agar media were prepared under laminar air flow bench. After solidifying the agar plates loop full culture was taken, which were prepared before 24 hrs of experiment. Whole plate streaking were done. Earlier mentioned each organism was taken and streaked in separate plate. each plate with the organism. Extract was prepared accordingly 1.0g/ml. With the help of sterile cork borers 8mm wells were punched in agar plates. Two wells were punched in each agar plate. one for *H. rosa-sinensis* extract and another for *Calendula officinalis*. 50mg of each flower extracts were poured in wells of each plate. Simultaneously each mentioned organism was streaked in separate agar plates and one well was punched in these agar plates.20 ul ampicillin was poured in these wells. These plates were used

as positive control. All experimental and positive control plates were placed in BOD incubator for 24 hrs. at 37°C. The whole experiment was performed under strict aseptic conditions.

## RESULT AND DISCUSSION

Extract of *H. rosa-sinensis* shown zone of inhibition against *Staphylococcus aureus* , *Salmonella typhimurium* ,*E.coli* ,*Proteus vulgaris*, *Pseudomonas aeruginosa*. Zone diameter shown in Table 1. *Staphylococcus aureus* showed maximum zone of inhibition (See Fig.1&2). *Staphylococcus aureus* causes nosocomial infection ,blood infection ,pimple ,boils and other skin infection ,scarlet fever etc. It also acquired resistance for most of the antibiotics. Flower extract of *H. rosa-sinensis* also inhibit the growth of other disease causing bacteria. This indicate that flower extract have potential for treating infection caused by *Staphylococcus aureus* and other clinical isolets. Flower extract of *Calendula officinalis* shown zone of inhibition against *Staphylococcus aureus*, *Bacillus subtilis*, *E.coli*, *Salmonella thyphi* . Zone diameter shown in Table 2. *E.coli* shown maximum zone of inhibition (see Fig.3&4) by *Calendula officinalis*. Followed by *Staphylococcus aureus*. Although I have also tried smaller concentration such as 10 and 25 mg/well ,but I didn't get any notable inhibition on bacterial growth. So I have used 50mg/well for better results.

## CONCLUSION

Our study concluded that plant extracts have antimicrobial potential. These flower patels contains flavonoids ,cyanidine ,quercetin ,calcium oxalate ,thiamine , ribofalavin ,niacin,ascorbic acid and oxalic acid(shukla and mishra 2001).Further biochemical and pharmacological investigation would be required for medicinal use.

Table 1.Antibacterial effect of Hibiscus rosa- sinensis flower extracts:

Amount of extracts in well - 50mg

S.No.	Name of Bacterial Isolates	Diameter of zone of inhibition(mm)
1.	<i>Staphylococcus aureus</i>	23
2.	<i>Salmonella typhimurium</i>	10
3.	<i>Escherichia coli</i>	11
4.	<i>Proteus vulgaris</i>	17

5.	<i>Pseudomonas aeruginosa</i>	20
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Table 2. Antibacterial effect of *Calendula officinalis* flower extracts:

Amount of extracts in well - 50mg

S.No.	Name of Bacterial Isolates	Diameter of zone of inhibition(mm)
1.	<i>Staphylococcus aureus</i>	14
2.	<i>Salmonella typhi</i> .	10
3.	<i>Escherichia coli</i>	18
4.	<i>Bacillus subtilis</i>	13
5.	<i>Pseudomonas aeruginosa</i>	14

Fig.1. Antibacterial effect of *Hibiscus rosa-sinensis* flower extracts:

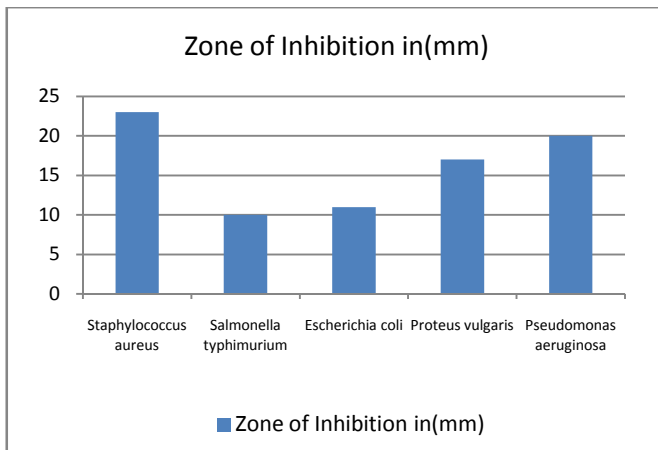


Fig. 2. *Staphylococcus aureus* shown maximum zone of inhibition

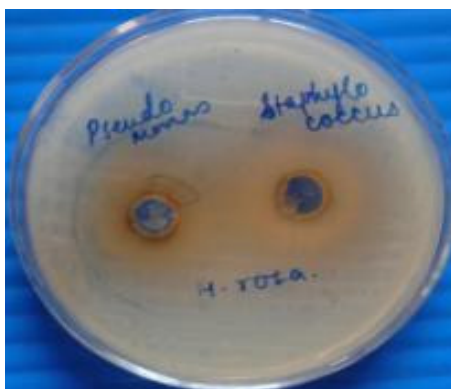


Fig. 3. Antibacterial effect of *Calendula officinalis* flower extracts:

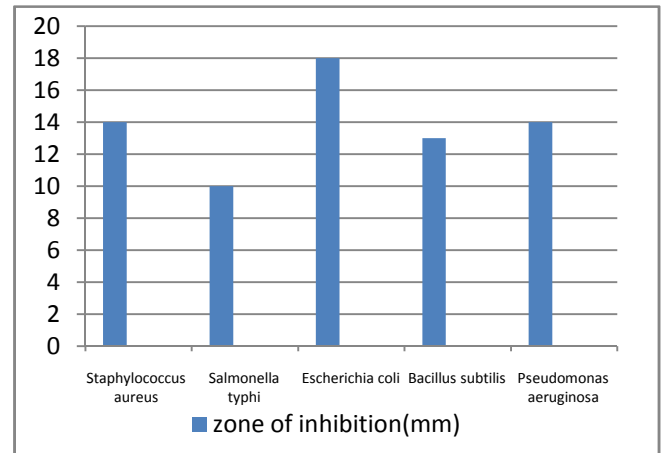


Fig.4 *E.coli* shown maximum zone of inhibition



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