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-“Study of antibacterial activity of *Rosa damascene* by using petal extract against different bacterial strains”

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ABSTRACT

Antimicrobial agents are substances that inhibit gram-positive and gram-negative microorganisms, preventing their growth. Antibacterial agents are categorized according to their mode of action, antibacterial agent source, and spectrum range. Roses come in a broad range of hues, which adds to their attractiveness. Many components of the rose have been tested for antibacterial and antifungal properties, and they are highly active in removing toxins from the body and have showed good results in inhibiting bacterial development. Different components of the rose can be utilized to fight with microorganisms in different ways. The flavonoid and phenolic components in rose extract are responsible for its antibacterial activity. The rose petals are powdered and mixed with methanol. Through agar disk diffusion method pure cultures were swabbed on Nutrient Agar and disk was placed on each plate and incubated at 37°C for 24 hours. Next day zone of inhibition was observed. To compare the activity of rose extract with antibiotic Analysis showed that when comparing the antibacterial activity of rose petals with control antibiotics, the zone of inhibition was higher to antibiotics at the higher concentration pure cultures were swabbed on MHA and antibiotic disk was placed on each plate. Next day zones were observed. Rose extract has shown maximum inhibitory effects against *E. coli* whereas the inhibitory effects against *Bacillus subtilis*.

KEYWORDS: Antibiotics, Antibacterial, Inhibition, Swabbed

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INTRODUCTION

The Rosaceae family includes roses. Roses come in a hundred different varieties (n=100). Roses are mostly found in the Northern Hemisphere's temperate zones. Roses exist in a broad range of color, which enhance their beauty. Flavonoids are natural plant chemicals that are generally secondary metabolites of plants having phenolic structures of various types¹. They have a variety of qualities that contribute in the treatment of cancer and Alzheimer's disease, including biochemical and antioxidant activities, antimutagenic and anticarcinogenic effects, etc.² They are categorized as a low molecular weight molecule and are derived from plants. The main source of flavonoids is rose and onion.³ Flavonoids, in addition to their antibacterial and antifungal effects, are also responsible for the color and smell of flowers. Terpene is a natural chemical found in

rose flowers. Terpenes are widely used in industry and pharmaceuticals. It's utilized as a flavouring or fragrance agent in the food industry. Terpenes have therapeutic use in the pharmaceutical industry.

Rose and its products have also been the subject of several research. Rose products and rose extracts have been proven to be effective in the treatment of a variety of diseases. The antiviral activity of rose flower was investigated in a recent study. Rose petals extract was produced with methanol and acetone separately in this study. This extract is now being used to treat HIV. Antiviral activity of rose petals extract against HIV was discovered. By interfering with HIV replication at several stages, it suppresses its activity. Kaempferol, a flavonoid found in rose petals, stops viral proteases from functioning⁴. Rose extract is also used to treat bacterial infections caused by a variety of germs. Both gram-positive and gram-negative bacteria are susceptible to it. However, many rose species, like the Bulgarian rose, have no antibacterial action.⁵ Rose extract was also found to have antioxidant properties. Rose's antioxidant action is due to phenolic compounds and flavonoids. The cancer prevention agent movement of rose basic oil is linked to the benefits of rose fundamental oil against formaldehyde inward breath on the reproductive system.

MATERIALS AND METHODS

The work was designed to study the antibacterial activity of *Rosa damascene*

Microorganisms There are two microbes were used to assess the antibacterial properties are gram positive and gram negative bacterial

Collection of Plant material :The fresh petals of rose were collected from garden (**Petal of *Rosa damascene***)

Solvent system for extractionIn this study two solvent system acetone and methanol were used for plant extraction

Processing of plant material :The fresh petals were surface sterilized simply by washing under tap water. and dried in shed for 20 days. After drying, petals were grounded in a grinder mixer to a powdered form and stored for further use.

Preparation of plant extracts: To study antibacterial activities of medical plant, it is very important to extract chemical component of our interest. *Rosa damascene* Petals Preparation of plant extracts for preparing the plant extracts, organic solvents, methanol (70%) were used. The powder of petals 30gm were extracted in a round bottom flask in 80 ml solvent (methanol). They were then kept in for 2-3 days in dark room at room temperature wrapped with aluminum foil to avoid evaporation. After 2-3 days, mixtures were filtered through What man No.1 filter paper, and filtrate was kept in incubator at 37 OC till all solvents completely evaporated from mixtures. Aqueous extract was prepared in 100mg/ml, 200mg/ml, 400mg/ml, and 800mg/ml concentrationThe rest of the powdered extract was weighed and dissolved in 500mg/ml DMSO. It was further stored at 4-degree Celsius after collecting in Eppendorf until use

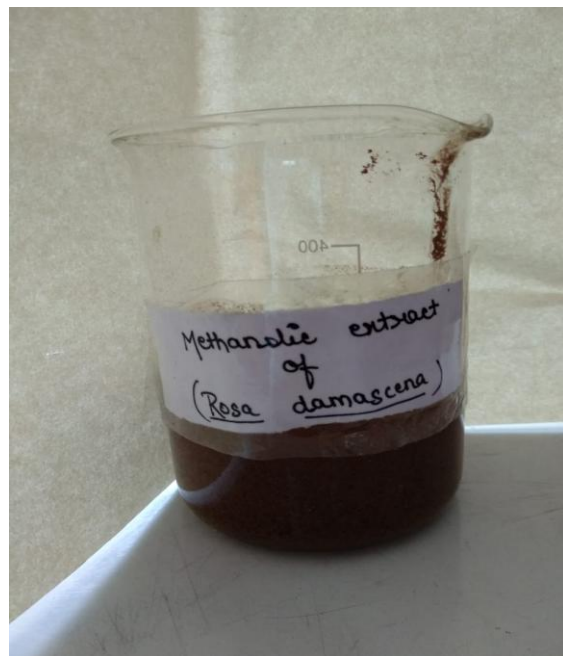


Fig:1 Methanolic extract of (*Rosa damascene*).



Fig:2 Rose Extract

Agar plate preparation for sensitivity test: 500ml of nutrient agar prepared and autoclaved at 121°C for 15 min. The agar was allowed to cool to 62°C in a water bath before pouring into petri dish

Disc Preparation Whatman No. 1 filter paper was used to make the 6mm (diameter) discs, which were then autoclave sterilised at 121°C. After the sterilization the moisture discs were dried on hot air oven at 50°C. Then various solvent extract discs and control discs were prepared.

Disk diffusion method

for primary selection of therapeutical agent disk diffusion method is used. The antibacterial activity of rose petals was tested against the two pathogenic bacteria *Escherichia coli*, *Bacillus subtilis* These two bacterial cultures were collected from Microbial Culture. These cultures were subculture on NA slants and stored at 4°C till use. For testing, the inoculums were prepared from the stock culture, which were sub cultured into nutrient broth (30ml) using a sterilized wire loop and incubated overnight in a rotary shaker at 37-degree C. After overnight incubation, these inoculate were further stored at 4 degree C till use. Antibiogram analysis (Disc Diffusion Assay) The antibacterial activity of Rosa damascene petals was evaluated against two pathogenic bacterial strains by using agar disk diffusion method under sterilized conditions. The test microorganism is inoculated and media was poured into the plates for the plates were prepared for all plant extracts. 1ml inoculum of each selected bacterium was uniformly spreader over Nutrient plates with the help of sterilized glass spreader. After five minutes four pieces of 6 mm disc previously impregnated with different concentrations of herbal extracts were placed onto the agar surface with sterile forceps. four different concentrations of crude extract (100mg/ml, 200mg/ml, 400mg/ml, and 800mg/ml) of DMSO and antibiotic disk were placed. The plates were incubated at 37°C for 24 hrs. in incubator. The results were observed on the next day and the effectiveness was measured in terms of diameter of zone of inhibition (clear zone).

Anti-bacterial activity: Zone of inhibition

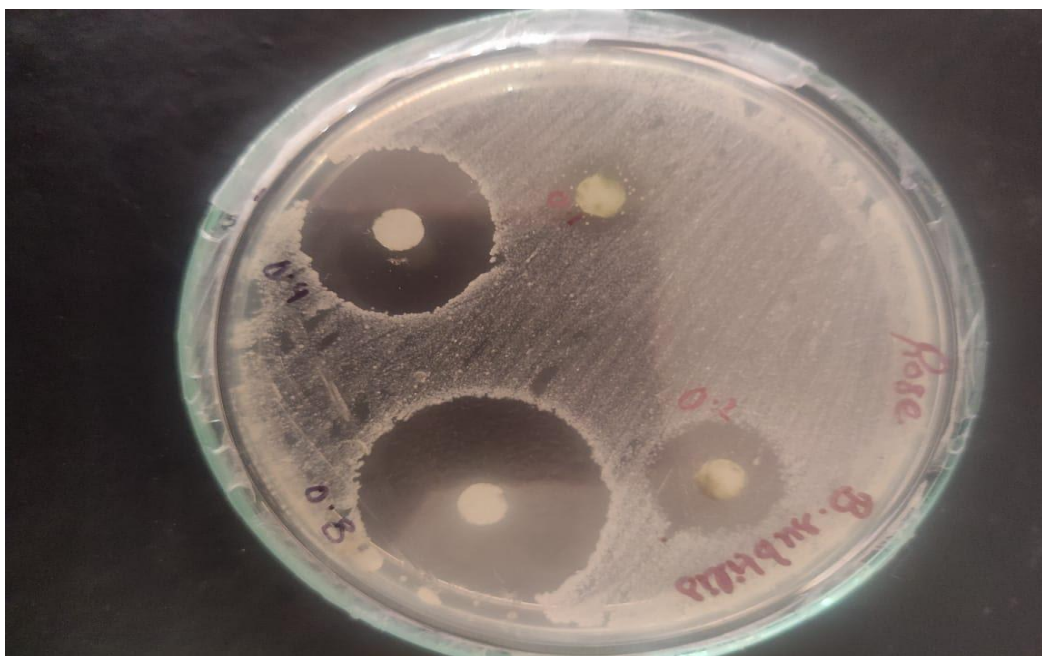


Fig:3 depicts zone of inhibition of rose petal extract at different concentration against *B. subtilis*

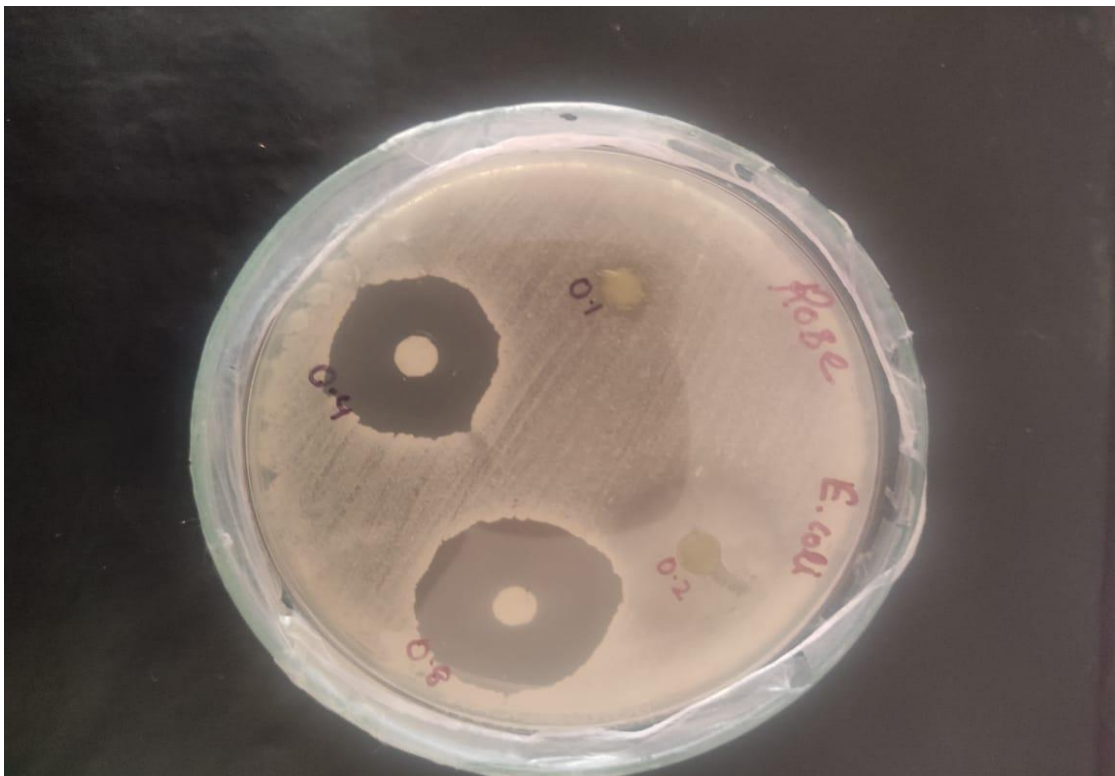


Fig: 4 depicts zone of inhibition of Rose extract at different concentration against *E.coli*

RESULTS AND DISCUSSION

Because of their resistant abilities, pathogenic microbes are the leading cause of infectious disease globally. The most significant need now is the identification of natural agents with the ability to kill or restrict the growth of microorganisms while having no harmful effects on humans. To fulfill the need for natural chemical discovery, several methods of study have been carried out. Traditional uses of plants as medicines offer a platform for determining which plant extracts may be beneficial for certain medical problems. Many plant extracts, such as rose extract, tea extract, and clove extract, have been utilized as antibacterial agents in the past, and they may offer to cure a variety of illnesses. It is critical to determine how natural plants with antibacterial activity can be utilized to treat a wide range of diseases or not. Several researches have been done to determine the antibacterial activity of various plant extracts in order to determine they are useful in the treatment of various illnesses or not

The antibacterial activity of rose extract was investigated in this work. This plant includes several components that impact human health, such as terpenes, glycosides, flavonoids, and anthocyanins. *R. damascene* has extensive pharmacological effects. This plant has a wide range of effects, including respiratory, cardiovascular, purgative, antidiabetic, antibacterial, HIV-resistant, relaxing, and cancer-prevention. The rose extract had the best antibacterial efficacy against *E. coli* and *Bacillus subtilis*, according to the research. But rose extract also showed activity against other tested microbes.

We used an antibiotic to compare the antibacterial activity of rose extract. antibiotic that works well against drug-resistant bacteria. It worked against all of the strains that were tested.

Table:1 shows inhibition zone diameters of Rose extract against bacillus subtilis and E.coli .

Sample	Solvent	Zone of inhibition (mm)									
		<i>Bacillus subtilis</i>					<i>E. coli</i>				
<i>Rosa damascene</i>	Methanol	100	200	400	800	positive	100	200	400	800	Positive of
		mg/ml	mg/ml	mg/ml	mg/ml	control of (Gentamicine)	mg/ml	mg/ml	mg/ml	mg/ml	Control (tetracyclin)
		7	11	16	17	25	8	12	13	16	27
		mm	mm	mm	mm	mm	mm	mm	mm	mm	mm

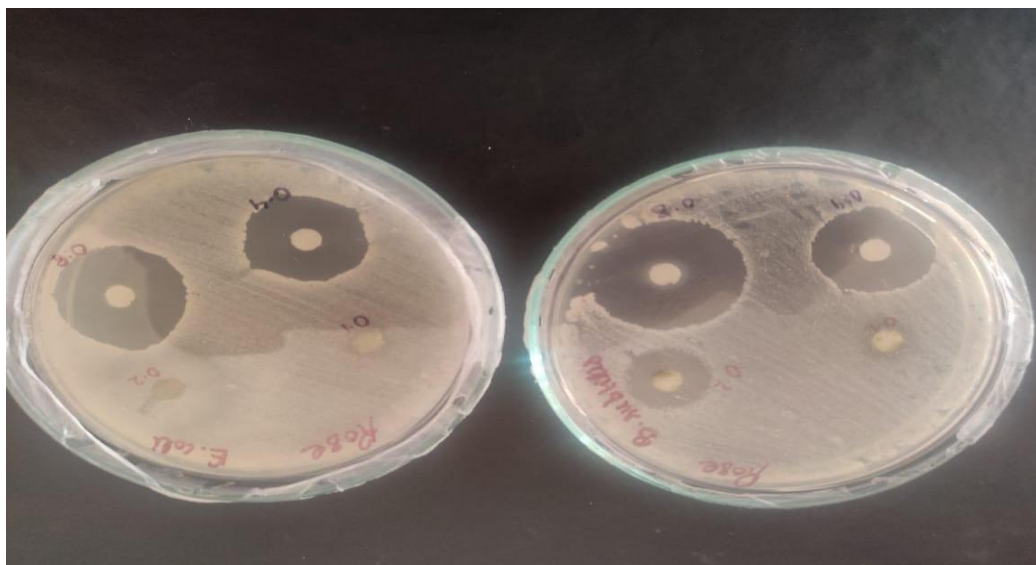
Fig:5 zone of inhibition against *E. coli* and *B. subtilis* of different concentration.



Fig:6 the diameter of the zone of inhibition correlates to the sensitivity of a bacterium *E.coli* to tetracycline (control plate)

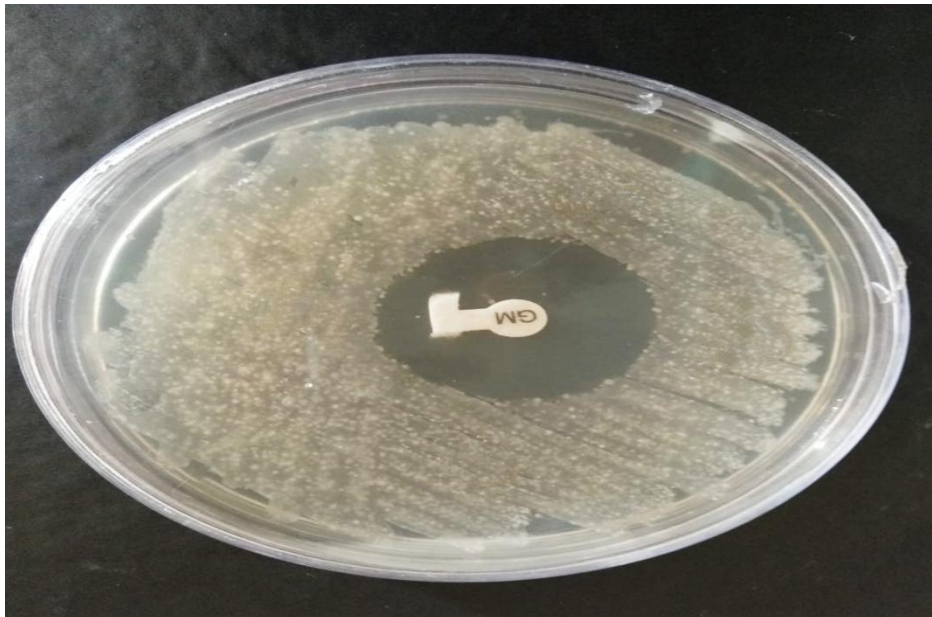


Fig:7 the diameter of the zone of inhibition correlates to the sensitivity of a bacterium *Bacillus subtilis* to Gentamicine (control plate)

CONCLUSION

According to the results of this study, all of the concentration extracts examined exhibited considerable antibacterial activity and may be utilised as a natural antibacterial source for treating drug-resistant bacteria. At a dosage of 800 mg/ml, rose extract appears to have the most antibacterial action.

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