

Antibacterial and Antifungal Efficacy of Rose Petal (*Rosa Indica*) Extract on Oral Microbes - An *In Vitro* Study

Swetha R^{1*}, Shobana R², Sunayana M², Prabu D², Raj Mohan² and Naveen Raj²

¹Department of Public Health Dentistry, Government Dental College and Hospital, Kadapa, AP, India

²Ragas Dental College, Chennai, India

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*For Correspondence:

Swetha Rajendra, Department of Public Health Dentistry, Government Dental College and Hospital, Kadapa, India, Tel: 9962535536

E-mail: swerajchand@gmail.com

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ABSTRACT

Introduction: Mother Nature has gifted with many herbs that have been found to have bactericidal and fungicidal action and these have been used from ages. One among them is Rose which is easily available everywhere like other herbs. Many diseases of human body are bacterial and fungal infections. Especially in oral cavity all the immune compromised patients are affected with fungal and bacterial infections. Use of these synthetic drugs may result in side effects and antibiotic resistance. So, the present study is all about determining the antibacterial and antifungal effect of rose. Hence, determining its application in dentistry, these can be used as an alternate to synthetic drugs to cure diseases.

Material and methods: Petals of *Rosa indica* were used for the preparation of ethanolic extract. 100 g of the powdered sample were soaked in 300 ml of Ethanol in a soxhlet apparatus. The sample obtained from the soxhlet apparatus was then dissolved in (dimethyl sulfoxide) DMSO. This is used to detect Antimicrobial activity of *Rosa indica*. Both zone of inhibition and minimum inhibitory concentration (MIC) were evaluated using Agar well diffusion method and micro-dilution method respectively. The results thus obtained were then compared with the control against *Streptococcus mutans* and *Candida albicans*.

Results: Zone of inhibition of rose extract was found to be 18 mm, 16 mm, 10 mm at 1000 µg, 500 µg, 250 µg against *Streptococcus mutans*, which was less than the control kanamycin (24 mm) and zone of inhibition was 26 mm, 24 mm, 22 mm at 1000 µg, 500 µg, 250 µg against *Candida albicans* which was greater than the control ketaconazole (19 mm). Thus, the rose extract has both antibacterial and antifungal property. Antifungal effect is more than antibacterial effect of rose.

Discussion: Zone of inhibition of rose extract is more when compared to ketaconazole, an antifungal drug and less than kanamycin, an antifungal drug. Rose extract has both antibacterial and antifungal property. Antifungal effect of rose is more than antibacterial effect and it is very effective against fungal diseases.

Conclusion: We can state that roses have both antibacterial and antifungal activity. In the present study rose extract has more antifungal property than antibacterial property. With this finding, rose can be used as a potent antifungal drug to treat fungal infections.

INTRODUCTION

Plants have always been considered over synthetic drugs from the past, as these have variety of chemical compounds to fight against viruses, bacteria, fungi and also against incurable diseases like cancer. Synthetic medicines are often used in treatment of infectious diseases. Even the diseases in oral cavity are treated with synthetic drugs. Synthetic drugs used for curing the diseases will result in side effects. Most common side effect ruled out among individuals is antibiotic resistance. Unlike synthetic drugs the use of herbal plants has less or no side effects and has no antibiotic resistance. WHO estimates 80% of population of African and Asian countries presently use herbal medicine for some aspect of primary health care and also regard them as the best source to variety of drugs^[1].

Among these herbs, rose has got many medicinal benefits. Rose is a woody perennial of Genus *Rosa*, within family Rosaceae. Most are native to India, with small numbers native to Africa, Europe and North America. These are mostly known for their beauty and fragrance. The word 'Rose' comes from Latin word 'Rosa'. Mostly rose petals, rose hip, seeds, rose water, leaves, oil and tea are used commercially to cure various infections^[2-5].

Rose acts against bacterial infections and relieves skin irritation and purifies it. It improves blood circulation and promotes hair growth, as well as controls dandruff. These are effective in soothing tired and fatigued eyes. Drinking rose water or tea removes inner body heat and brings down fever. It acts as an antiseptic, antispasmodic, astringent, bactericidal (against typhoid, diarrhoea, cholera, food poisoning), antiviral (cold or influenza), antiphlogistic. It helps to remove scars and marks. In the treatment of dreadful cancers patients are often subjected to severe anti-cancer medicines, and results in immune-suppression. In these patients opportunistic infections are more predominant. Rose extract contains 80% essential fatty acids and antioxidants, thus helps to regenerate skin cells. In case of oral diseases, fungal infections mainly candidiasis is often use of rose extract in curing the diseases is much effective. According to studies done in 2007 and 2013 found that roses have high levels of polyphenols^[6,7]. A 2005 study on polyphenols published in American journal of clinical nutrition found that polyphenols could potentially help to prevent cardiovascular diseases, osteoporosis and deadliest disease like cancer^[2-4]. It helps to increase the activity of immune system^[1-11].

Oral bacteria like *Streptococcus mutans* and *Lactobacillus* are the main microorganisms implicated in the initiation and progression of caries, respectively. Immune-suppression allows the formation of mucosal biofilms, leading to the clinical appearance of thrush^[12].

With reference to study published in the year 2012 proves that rose contain vitamin C^[13]. It also contains vitamin B1, B2, B3, K, E, sugars, flavonoids and tannins. It strengthens tooth and gum. It produces beneficial effects in toothache. Powder of rose is sprinkled in mouth to treat stomatitis. Hence, roses are of great use in dentistry as it proves to cure paradontosis. Rose when used as a mouthwash reduces bad odour^[2,3,10,11,14-16].

This study was done to deeply investigate the antibacterial and antifungal action of rose and its action on various dental infections. By proving so, roses can become a vital part of dentistry in the near future and extensive studies on its components and properties will enable us to utilize its benefits.

MATERIALS AND METHODS

Samples

Rosa indica flowers were collected from market identified by a horticulturist. The flowers were sundried and were diluted in 30 ml of ethanol.

Pathogens

Two different pathogens namely *Streptococcus mutans* and *Candida albicans* available at MRD Life Sciences (P) Ltd. Lucknow, collected from Institute Of Microbial Technology, (IMTECH), Chandigarh, were subcultured and used throughout the project work.

Preparation of crude ethanolic extracts

Ethanolic extracts were extracted by 100 g powder, soaked in 300 ml of Ethanol in a soxhlet apparatus. The above preparation was repeated thrice. Ethanolic extracts condensed with rotary evaporator with constant temperature 45°C and made powder, dissolved in dimethyl silfoxide (DMSO) solvents and used for antimicrobial activities.

Well diffusion method

Fresh bacterial culture of *Streptococcus mutans* MTTC 890 and fresh fungal culture *Candida albicans* MTTC 773 were used for the antimicrobial test. Fresh microbial cultures were used for the antimicrobial activity. The colonies of the strains were inoculated to Muller Hinton broth for bacteria and Potato Dextrose Broth (PDB) for fungi and incubated at 37°C for 24 h in orbital shaker at 200 rpm. Turbidity was adjusted with sterile broth to corresponds to the 0.5 McFarland standards before swabbing; standard inoculum of the microorganism was of 1.5×10^6 Colony Forming Units (CFU mL⁻¹) diluted to 1:100 and given suspension of turbidity equal to a McFarland standard 0.5. The turbidity was adjusted to match a McFarland 0.5 mL of 1.175% w/v (0.048 M) BaCl₂.H₂O to 99.5 mL of 1% w/v (0.36) sulphuric acid.

Standard antibiotic kanamycin for *Streptococcus mutans* and fluconazole for *Candida albicans* was used as reference. Organisms (24 h old culture) were swabbed on the Muller Hinton Agar (MHA) to bacteria and potato dextrose agar (PDA) for fungi plates with sterilized cotton swab sticks. Wells (9 mm diameter) were cut using a sterile cork borer. Stock solutions of ethanol extracts of rose powder was dissolved in dimethyl sulfoxide (DMSO). The stock solution was prepared with to 10 mg/ 2 ml of DMSO. From the stock solution, different diluted measurements such as 50 µl, 100 µl, 200 µl (10 µl diluted sample contain 50 µg of the test compound) were immediately dispensed into agar wells of culture inoculated plates (MHA) using sterilized micro tips. The plates were incubated at 37°C overnight. The antibacterial activity was measured as the diameter of the inhibition zone including the diameter of the well and inhibition percentage was calculated using the following formula:

Anti-Microbial Activity: Zone of Inhibition

Figure 1 depicts zone of inhibition of Kanamycin and rose petal extract at different concentration in Muller Hinton agar swabbed with *Streptococcus mutans*. Zone of inhibition of Kanamycin is more than the sample.

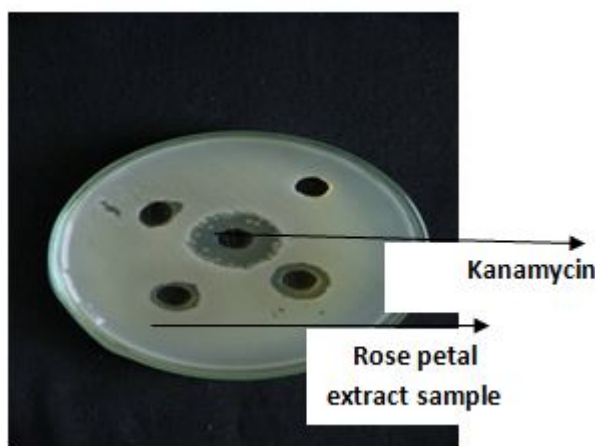


Figure 1. Antibacterial activity of rose powder against streptococcus mutans.

Figure 2 depicts zone of inhibition of Ketoconazole and Rose petal extract at different concentration in Potato Dextrose agar swabbed with *Candida albicans*. Zone of inhibition of the sample was more than Ketoconazole.

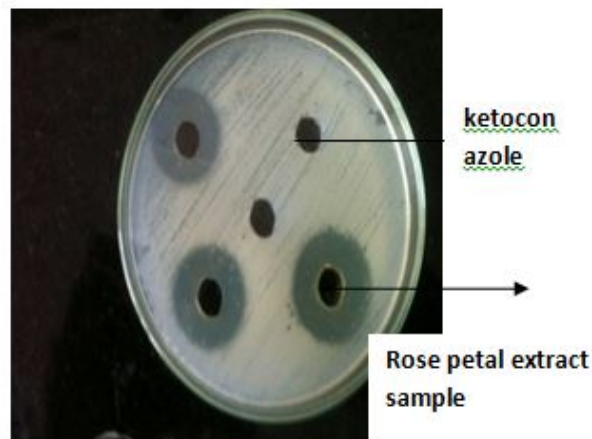


Figure 2. Antifungal activity of rose powder against *Candida albicans*.

Minimum Inhibitory Concentration (MIC)

MIC was determined by micro-dilution method using serially diluted (2 folds) rose extracts according to National Committee for Clinical Laboratory Standards, (NCCLS) 2000. Ethanolic extracts which showed significant zones of inhibition were chosen to assay. Muller Hinton broth was prepared and sterilized using autoclave. 100 μ l of the prepared broth was dispensed in to the well numbered 1-12 using sterile micropipette. A stock solution containing mg/ml of the extract was prepared. Then 100 μ l of the solution was dispensed into the tubes numbered 1.

Subsequently, from well 1, serial dilution was carried out and 100 μ l from tube 1 was transferred up to well number 10 and 100 μ l from the well 10 was discarded. Well 11, and 12 has viability of the organism control. An overnight culture (inoculums) of each of the test isolates was prepared in sterile nutrient broth. 100 μ l of the inoculum was transferred into each tube from well 1 to 12. The final concentration of the ethanol extract in each of the plate well numbered 1-10 after dilution 500 μ g/ml 250 μ g/ml; 125 μ g/ml; 62.5 μ g/ml; μ g/ml 31.25 μ g/ml; 15.625 μ g/ml; 7.812 μ g/ml; 3.90 μ g/ml; 1.953 μ g/ml and 0.976 μ g/ml were incubated at 37°C for 24 hrs and examined for growth. Subsequently, each well with 20 μ l of Blue tetrazolium (5 mg/mL) freshly prepared in PBS buffer and incubated for 30 min. After incubation, well showed blue color indicate viability of organisms and presence of yellow color or absent of blue color indicate complete inhibition of bacteria and fungi.

Equal volume of extract and nutrient broth were mixed in a test tube. Two control tubes were maintained for test batch. These included antibiotic control (tube containing extract and growth media without inoculum) and organism control (tube containing growth medium, saline and inoculum). The lowest concentration (highest dilution) of the extract that produced no visible bacterial growth (no turbidity) when compared with the control tubes were regarded as MIC.

With the use of serial dilution method minimum inhibitory concentration of rose powder was determined. It was found that minimum inhibitory concentration of rose powder against *Streptococcus mutans* was at 6 (15.63 μ g) and *Candida albicans* was at 6 (15.63 μ g).

RESULT

Zone of Inhibition

Zone of inhibition is the degree of sensitivity of the bacteria to a drug. Antimicrobial activity of the rose sample was determined by agar well diffusion method (Figures 3 and 4).



Figure 3. Minimum inhibitory concentration (MIC) of rose sample extract and kanamycin using microdilution method. A: Rose powder (MIC at 6); B: Kanamycin (MIC at 9); C: Tetracycline (MIC at 9) 1. 500 µg; 2. 250 µg; 3. 125 µg; 4. 62.5 µg; 5. 31.25 µg; 6. 15.625 µg; 7. 7.812 µg; 8. 3.90 µg; 9. 1.953 µg; 10. 0.976 µg; 11 and 12. Organism control.

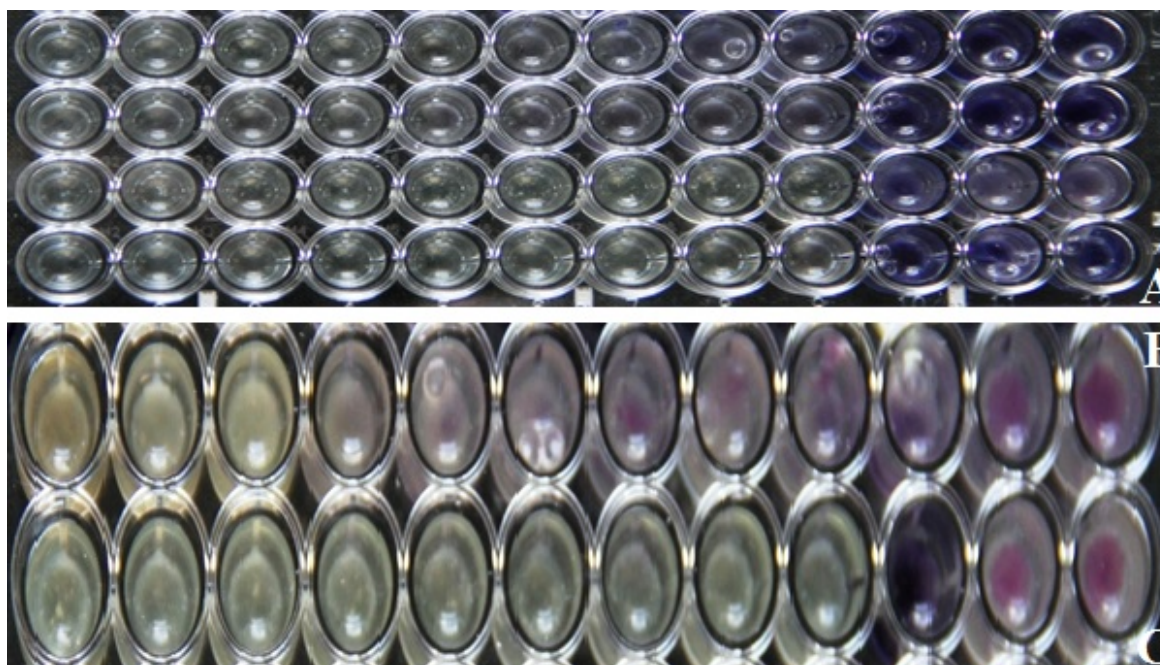


Figure 4. Minimum inhibitory concentration(MIC) of Rose sample extract and ketoconazole using Microdilution method. A: Rose powder (MIC at 6); B: Fluconazole (MIC at 4); C: Ketoconazole (MIC at 9) 1. 500 µg/mL; 2. 250 µg/mL; 3. 125 µg/mL; 4. 62.5 µg/mL; 5. 31.25 µg/mL; 6. 15.625 µg /mL 7. 7.812 µg; /mL 8. 3.90 µg/mL; 9. 1.953 µg/mL; 10. 0.976 µg/mL; 11 and 12. Negative control.

Table 1 shows the results of zone of inhibition observed for the antimicrobial extracts and Standard antibiotic kanamycin. When 1000 µg, 500 µg, 250 µg of the sample was taken which showed 18, 16, 10 mm zone of inhibition

respectively. This suggests that Antibacterial activity increases with increase in the concentration of the sample. But kanamycin has better zone of inhibition than rose petals and which is 24 mm.

Table 1. Illustrates the reaction of *Streptococcus mutans* to the concentration of the sample.

Microbes	Concentration			
S. mutans	1000 µ g	500 µ g	250 µ g	Kanamycin
Zone of inhibition (in mm)	18 mm	16 mm	10 mm	24 mm

Table 2 shows the results of zone of inhibition observed for the antimicrobial extracts and the standard Antifungal ketoconazole. When 1000 µ g, 500 µ g, 250 µ g of the sample was taken showed 26, 24, 22 mm zone of inhibition respectively. This suggests that Antifungal activity increases with increase in the concentration of the sample. Antifungal activity of rose petal extract is more than ketoconazole which is only 19 mm.

Table 2. Illustrates the reaction of *Candida albicans* to the concentration of the sample.

Microbes	Concentration			
Candida albicans	1000 µ g	500 µ g	250 µ g	Ketoconazole
Zone of inhibition (in mm)	26 mm	24 mm	22 mm	19 mm

DISCUSSION

There are many studies describing the antibacterial or antifungal effect of *Rosa indica* but very less compares both these effects and their extent of action. In the present study, both the effects of *Rosa indica* were compared and were found that it has better antifungal property than its antibacterial property.

Zone of inhibition indicates size of this zone depends on how effective the antibiotic action is in stopping the growth of bacteria. In the present study, zone of inhibition of ethanolic Rose extract against *Streptococcus mutans* was found to be 18 mm, 16 mm, 10 mm at 1000 µ g, 500 µ g, 250 µ g respectively and that of control (Kanamycin) is 24 mm. This shows that Rose petals have less antibacterial potency than Kanamycin (Antibacterial drug). The finding of present study with regard to antibacterial activity of Rose petal sample is in contrary to that of the study conducted by Aslam et al. [8] which shows the ethanolic extract of *Rosa indica*, zone of inhibition is 26, 25, 27 mm against *Staphylococcus aureus*, *Psuedomonas aeruginosa*, *Escherichia coli* respectively where the control Tetracycline has zone of inhibition of 20, 26, 17 mm [13]. This shows antibacterial activity is more than Tetracycline. These findings can state that *Rosa indica* has more antibacterial property against *Staphylococcus aureus*, *Psuedomonas aeruginosa*, *Escherichia coli* and has less antibacterial effect on *Streptococcus mutans*. When ethanolic extract of *Rosa indica* were used against *Streptococcus mutans* the antibacterial effect is less as compared to Kanamycin as control. This is similar to a study conducted by Hirulkar et al. [9] where he used ethanolic extract of rose petal against *streptococcus pneumonia* showed zone of inhibition of 27 mm which has more antibacterial activity when rose petals are extracted from petroleum ether where Zone of inhibition of *Streptococcus pneumonia* showed zone of inhibition of 16 mm, this states that ethanolic extract is more effective than petroleum ether extract with regard to *Rosa species* [9].

In the present study zone of inhibition of rose extract against *Candida albicans* was 26 mm, 24 mm, 22 mm at 1000 µ g, 500 µ g, 250 µ g respectively and that of Ketoconazole is only 19 mm. This shows potent antifungal property of rose extract which is effective than Ketoconazole. A study by Takte et al. [17] states that Methanolic extract of *Rosa damascena* showed Zone of inhibition of 8.3, 14.9, 21 mm at 20, 40, 80 mg respectively against *Candida albicans* to that of Fluconazole control. This shows methanolic extract of *Rosa damascena* has more Antifungal activity against *Candida albicans*.

According to Saeed et al. [18] and Manjari et al. [19] antifungal effect of *Rosa indica* is due to the presence of phytochemicals like alkaloids, phenolic acids, flavanoids, tannins and volatile oils [18,19]. A study by Godstime et al. [20] showed that phytochemicals may act by inhibiting microbial growth, inducing cellular membrane perturbations, interference with certain microbial metabolic process, modulation of signal transduction or gene expression pathways.

Rosa indica exhibits antifungal than antibacterial property against bacterial and fungal strains which are present in the environment that cause serious infection. So, it can be used to cure opportunistic infections which are leading fungal infections.

CONCLUSION

Investigation shows that Extract of *Rosa indica* has potent antifungal activity than its antibacterial activity. This shows that it has a promising effect in curing fungal diseases like Candidiasis which occurs as an opportunistic infection in the oral cavity of HIV and other immunocompromised patients. Further research has to be performed *in vivo* to know its pharmacological action in curing fungal diseases. So, that it can become an alternative to the antifungal drugs. Even though these drugs cure fungal diseases it also causes adverse effects during its consumption. *Rosa indica* have been found everywhere and can become an easy source for patients with opportunistic infections and other infections. Mostly, roses are known for its beauty but have got therapeutic applications too. However, further pharmacological studies are needed to establish its medicinal value.

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