

## Determination of Antibacterial and Antifungal Properties of Rose Extract- An *In vitro* Study

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### ABSTRACT

Background: Rose is a perennial plant of the genus *Rosa*, within the family *Rosaceae*. Various studies have been conducted to establish the anti-fungal, anti-bacterial and anti-oxidant activities of rose. This in-vitro study was planned to determine the antibacterial and antifungal potential of rose against periodontal pathogens. Materials and methods: Rose petals were collected, dried and subjected to extraction with ethanol. The minimum inhibitory concentration of the extract was then determined against oral and periodontal bacteria and fungi. Results and conclusion: The rose extract was found to exhibit an appreciable antifungal activity and antibacterial activity against gram positive anaerobes than periodontal pathogens which are gram negative anaerobes.

**Keywords:** Rose, antibacterial, antifungal, periodontal pathogens.

### INTRODUCTION

Plant materials have been widely used for the treatment of diseases, and till date they play an important role to cover the basic health needs in developing countries. A variety of plant materials and phytochemicals have been found to exhibit effective antibacterial activity<sup>1,2</sup>. Rose is a perennial plant of the genus *Rosa*, within the family *Rosaceae*. Rose has influenced cultures artistically, economically, clinically, scientifically, psychologically and religiously because of its fragrance. There are approximately 10,000 species of rose; *R. damascene*, *R. gallica*, *R. centifolia*, *R. indica*, *R. rubiginosa* to name a few<sup>3</sup>. Rose is the most celebrated of all the fragrances, reason being the presence of anthocyanins in the petals. Various parts of the rose plant have been tested for their use. Rose petals and leaves work as a diuretic and flush toxins from the body. They are known to relieve bronchial and chest congestion and also provide relief from sore throat. Rose water because of its antiseptic property has been used as eyewash, and is also used to moisturize the skin<sup>4</sup>. Various studies have been conducted to establish the anti-fungal, anti-bacterial and anti-oxidant activities of rose. These activities were attributed to the presence of flavonoid and phenolic compounds, also known as bioactive agents<sup>5-8</sup>. However, hardly any reports are available on the evaluation of the extracts of rose against periodontal pathogens. Thus, this in-vitro study was planned to determine the antibacterial and antifungal potential of rose against periodontal pathogens.

### MATERIALS AND METHODS

The rose flowers were collected from a local market, and authenticated by an expert. From rose, the petals were

removed and subjected to air drying under shade for one week. Completely dried samples were powdered, weighed 200 g and subjected to extraction with ethanol 950 ml in the Soxhlet apparatus (20 cycles). Extraction was carried out at 75 °C temperature. After completion of the extraction, the extract was filtered through What man paper no.1 and concentrated under reduced pressure at 40 °C temperature using rotary evaporator. Extract was stored in desiccator for further use. Percentage yield of the extract was calculated to be 3.2 % w/w. The minimum inhibitory concentration of the extract against bacteria namely *Streptococcus sanguis*, *Streptococcus mitis*, periodontal pathogens (*Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans* and *Prevotella intermedia*) and fungi namely *Candida albicans* was determined. For determining the MIC, Brain Heart Infusion (BHI) broth was used as the medium. First tube contained 20 µl of the rose extract and 380 µl of thioglycollate broth. For further dilutions, 200 µl of thioglycollate was added into the next 9 tubes separately. From the initial tube, 200 µl was transferred to the first tube containing 200 µl of the broth. This was considered as 10<sup>-1</sup> dilution. 200 µl of the broth was then transferred from the 10<sup>-1</sup> dilution tube, to make it 10<sup>-2</sup> dilution. This serial dilution was repeated upto 10<sup>-9</sup> dilution. For the maintained stock cultures of organisms, 5 µl was taken and added into 2 ml of thioglycollate broth, and 200 µl of this suspension was then added to each serially diluted tube. These tubes were then incubated for 24 hours and observed for turbidity. For facultative anaerobes, tubes were incubated at 37 °C for 48-72 hrs in CO<sub>2</sub> Jar, and for strict anaerobes, tubes were incubated in anaerobic jars for 48-72 hrs<sup>9</sup>.

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Table 1: Minimum inhibitory concentration of rose extract against microorganisms.

Microorganisms	Rose extract ( $\mu\text{g/ml}$ )									
	100	50	25	12.5	6.25	3.12	1.6	0.8	0.4	0.2
<i>A. actinomycetemcomitans</i>	S	R	R	R	R	R	R	R	R	R
<i>P. gingivalis</i>	S	S	R	R	R	R	R	R	R	R
<i>P. intermedia</i>	S	S	R	R	R	R	R	R	R	R
<i>Candida albicans</i>	S	S	S	S	S	S	S	S	S	R
<i>S. mitis</i>	S	S	S	S	S	S	S	S	S	R
<i>S. sanguis</i>	S	S	S	S	S	S	S	S	S	R

S = sensitive, R = resistant

## RESULTS

The antibacterial and antifungal properties of ethanolic extract of rose petals were determined in the present study. It was noted that, for gram positive facultative anaerobes namely *S. mitis* and *S. sanguis* the extract was sensitive till the 9<sup>th</sup> dilution i.e till 0.2 $\mu\text{g/ml}$ . The extract was sensitive to *C. albicans* till 0.2  $\mu\text{g/ml}$ , thus displaying good antifungal and antibacterial properties. However, when the extract was tested against periodontal pathogens namely, *P. gingivalis*, *P. intermedia* and *A. actinomycetemcomitans*, it was seen to be sensitive at the 1<sup>st</sup> dilution i.e at 100  $\mu\text{g/ml}$ . The rose extract was thus found to exhibit a better antifungal activity and antibacterial activity against gram positive anaerobes than periodontal pathogens which are gram negative anaerobes (Table 1).

## DISCUSSION

Plant and plant materials have been widely used to cover health care needs, and this form of medicine is termed as herbal medicine, herbalism or herbology. Plant materials have been widely used for the treatment of infectious diseases and have gained popularity as they decrease the side effects seen with the use of systemic antimicrobials. Prior to using these plant and plant materials, a thorough screening of the plants should be done, which would help to isolate and characterize the active compounds. Rose and parts of the rose plant have been in use in medicine, the evidence for which dates back to the "Arkprakash" one of the vedas of Ayurveda<sup>10</sup>. Owing to its fragrance, rose has been seen to influence culture artistically, economically, clinically and scientifically. This fragrance of rose is essentially due to oil secreted in the papillae, especially geraniol, citronellol, ethanol, rose oxide, linalool, nerol, eugenol<sup>11</sup>. Various parts of the rose plant, including the stem leaves and the petals have been observed to possess antibacterial and antifungal properties. These properties are noticed in the active compounds that are extracted from the plant, and the extraction of the same depends on the solvent that is used for the extraction process. The most commonly used solvents are methanol and ethanol. Alcohols are used as, they extract both polar and non-polar constituents from plants<sup>12,13</sup>. Considering above benefits, in the present study ethanol was used as solvent for extraction. Antibacterial activity of rose has been demonstrated against various microorganism viz., *Staphylococcus aureus*, *Proteus mirabilis*, *Escherichia coli*, *Streptococcus pneumonia*, *Shigella flexneri*, *Pneumonia auregenosa*, *Salmonella typhimurium*, *Vibia*

*cholera*<sup>8</sup>. This antibacterial property could be due to the presence to the several phytochemical constituents like alkaloids, flavonoids, glycosides, saponins, tannins, steroids, anthraquinones, phenols, resins, fatty acids and gums<sup>8</sup>. The activity of rose against oral pathogens is not reported, and the present study is the first in that direction. In the present study, antibacterial activity was noted against *S. mitis*, *S. sanguis*, *P. gingivalis*, *P. intermedia* and *A. actinomycetemcomitans*. Also antifungal activity was noted against *C. albicans*. However, a pronounced antibacterial effect was exhibited against the gram positive bacteria than gram negative bacteria. This could be attributed to the fact that gram negative bacteria possess an outer membrane which acts as a barrier which prevents/reduces the penetration of antimicrobials. Lack of outer membrane in gram positive bacteria makes them more vulnerable to damaging molecules and this leads to leakage of their cytoplasmic contents<sup>14</sup>.

## CONCLUSION

The present study demonstrates the antibacterial and antifungal properties of the ethanol extract of rose petals against periodontal pathogens. This is one of the first studies evaluating the effect on periodontal pathogens. Further in detail studies on characterization of extract are needed for product development.

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