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DETERMINATION OF ANTIBACTERIAL ACTIVITY OF RUTA GRAVEOLENS AGAINST STREPTOCOCCUS GORDONII AND STREPTOCOCCUS SALIVARIUS – AN IN VITRO STUDY

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ABSTRACT

Background: Primary colonizers lead to the development of microcolonies and eventually to a mature biofilm in the oral cavity. Herbal medicinesare being used sincea long time in the treatment of periodontal diseases. Ruta graveolens, a medicinal plant belonging to Rutaceaefamily is reported to haveantimicrobial, antiviral, antipyretic, anti-inflammatory and analgesic properties. This studyaimed to determine the antibacterial activity of R. graveolens againstprimary colonizing bacteria Streptococcus gordonii and Streptococcus salivarius. **Materials and Methods**: Ruta graveolens plant was collected and processed using Soxhlet apparatus. Antibacterial activity of methanolic extract of R. graveolens was tested against S. gordonii and S. salivarius bacterial strains at four concentrations 15%, 25%, 40%, 50% using well diffusion technique. **Result**: R. graveolens extractshowed the largest zone of inhibition, 18.5 mm against S.gordonii and16.0mmagainst S. salivariusat 50 % concentration. And no zone of inhibition found for 15%, 25% and 40% concentrations against S. salivarius. **Conclusion**: It could beconcluded that methanolic extract of R. graveolens possess significant antibacterial activityagainst Streptococcus gordonii and Streptococcus salivarius.

KEYWORDS: Ruta graveolens, antibacterial activity, Streptococcus gordonii, Streptococcussalivarius.

INTRODUCTION

Gingivitis is a periodontal disease resulting from microbial infection and subsequent inflammation. Conventional periodontal therapy consists of oral hygiene instructions, scaling and root planning. However, some patients do not respond favourably to conventional therapy and adjunctive therapeutic agents may be necessary.Antimicrobial and non-steroidal anti-inflammatory drugs have been studied as an adjunctive to mechanical plaque biofilm control. Yet, use of these drugs are related to antimicrobial resistance, systemic alterations and gastrointestinal intolerance.[1] In the quest for safe and effective antimicrobial agent, phytotherapy is emerging as an alternative with reduced side effects. Various herbal medicines have been used as adjuvant in the treatment of periodontitis.[2,3] Ruta graveolens, commonly known as Garden Rue belongs to the family Rutaceae.[4] Studies about R. graveolens found that it contains a large amount of secondary metabolites such as volatile oils, flavonoids, coumarins and phenolic acids. These products could be responsible for anti‑inflammatory, analgesic, antimicrobial, antipyretic, and free radical scavenging activities.[5,6] R. graveolens is biologically valuable source offuranocoumarin andfluroquinolone alkaloids. Linear furanocoumarins have been used inpigmentation disorders, skin diseases, and neurological diseases.[7]

Primary colonizersof dental biofilm lead to adhesion of other oral bacteria by providing new binding sites. They modify the local microenvironment through their metabolic activities favouring survival of other bacteria in the dental biofilm. S. gordonii andS. salivarius are amongst important primary colonizers in dental plaque.[8,9] However the literature describing bioactivity of R. graveolens against S. gordonii andS. salivarius are scanty. Therefore, this studywas aimed to determine the antibacterial activity of R. graveolens against S. gordonii andS. salivarius.

MATERIALS AND METHODS

The Ethical Committeeof institutionapproved thepresent study.

Bacterial isolates and sample preparation: Clinically isolated strains ofS. gordonii CIP 110914 and S. salivarius CCHSS3 were employed in this study.S.gordonii and S. Salivariusstrains preserved at laboratory were sub-cultured 24 hours prior to the study.

Collection and processing of test plant material: R. graveolens was collected from Ethno Medicinal Garden, FRLHT, Bengaluru, on 29th February 2020 and authenticated by Dr. N.M.Ganesh Babu, Assistant Professor, The University of Trans-Disciplinary Health Sciences & Technology, Bangalore. R. graveolens was processed in the month of March.

R. graveolens was dried in hot air oven following thorough rinsing with sterile distilled water, crushed with electrical blender.

Extraction of plant material: The procedure for the preparation of methanolic extract will be carried at Sri Adichunchunagri college of Pharmacy, B.G.Nagara, Karnataka. The extraction of R. graveolens was prepared according to the procedure described by Salman HA et. al., using Soxhlet apparatus.[10] The extractwas filtered using Whatman paper no.2. Different concentrations (15, 25,40, 50 mg) of plant extracts were prepared andstored.

Antimicrobial activity assay: S. salivarius and S. gordonii were grown in Brain heart infusion liquid culture medium at 37℃ for 24 hours. Anti-bacterial activity was evaluated by well diffusion technique described by Tijjani MA et al.[11] 200µl of each sample (15%, 25%, 40%, and 50%) were added to separate wells in the culture plates and incubated at 37℃ for 24hours, following which, the diameter of the zone of inhibition was measured to nearest millimetre.

Statistical analysis

Present study statistical analysis was done using SPSS version 20 and Microsoft excel 2007. Statistical analysis included descriptive and inferential statistics. Descriptive statistics include mean and standard deviations. In Inferential statistics mean inhibitory zone at different concentration was compared using Kruskal Wallis ANOVA. And pairwise comparison was done using Mann-Whitney U test.

RESULTS

In present study, methanolic solvent extract of Ruta graveolens plant leaves was collected. The antimicrobial activity of Ruta graveolens extract was examined using well diffusion technique. S.gordonii and S. salivarius, 2 groups were taken and each group contain 3 samples. The inhibitory zone was measured in each sample. The mean inhibitory zone diameter of S.gordonii and S. salivarius at 15%,25%,40% and 50% concentrations were

compared using Kruskal Wallis ANOVA and pair wise comparison was done by Mann-Whitney U test.

The average zone of inhibition of R. graveolens against S. gordonii and S. salivarius at different concentrations is presented in Table 1. The test revealed that there was a statistically significant difference in mean diameter of inhibitory zone between 15%, 25%, 40%, and 50% concentration in S.gordonii group (i.e., p=0.021). There was a statistically significant difference in mean diameter of inhibitory zone between 15%, 25%, 40% and 50% concentration in S.salivarius group. (i.e., p=0.013).

In the present study pair wise comparison of mean inhibitory zone between groups was compared using Mann whitney U test. In S.gordonii group, there was no statistically significant difference in mean inhibitory zone between 15% and 25% (i.e., p=0.050), between 15% and 40% (i.e., p=0.050), between 15% and 50% (i.e., p=0.050), between 25% and 40% (i.e., p=0.261), between 25% and 50% (i.e., p=0.050) and between 40% and 50% (i.e., p=0.050).

In S. salivarius group, there was no statistically significant difference in mean inhibitory zone between 15% and 25% (i.e., p=1.000), between 15% and 40% (i.e., p=1.000) and between 25% and 40% (i.e., p=1.000), but there was statistically significant difference in mean inhibitory zone between 15% and 50% (i.e., p=0.037), between 25% and 50% (i.e., p=0.037) and between 40% and 50% (i.e., p=0.037) (Table 2).

Graph 1 and Graph 2represent antibacterial activity of Ruta graveolens against S.gordonii and S.salivarius respectively.

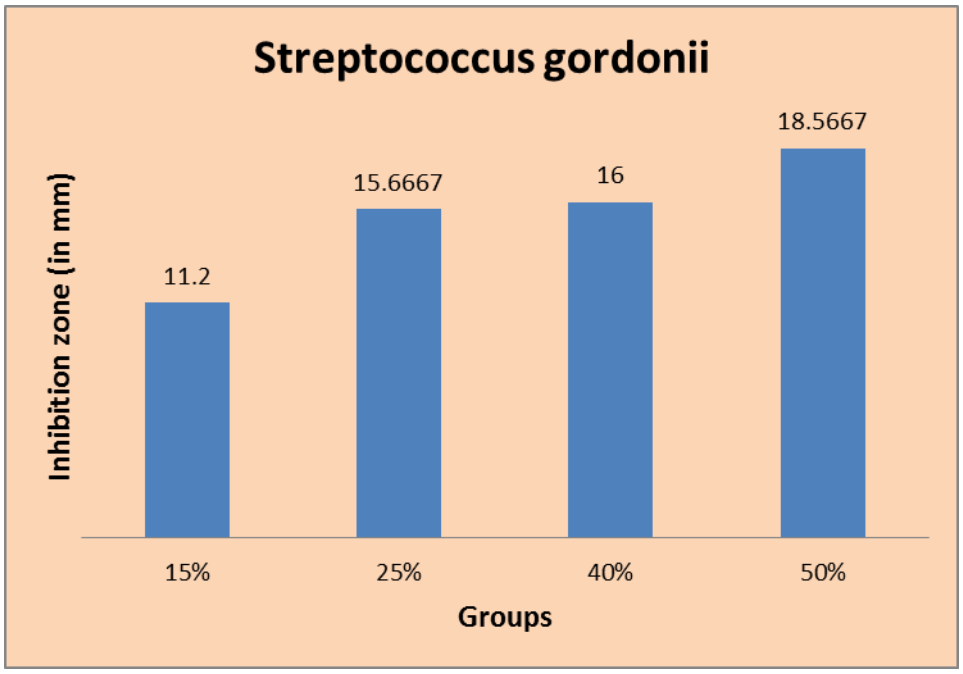
Table 1: Comparison of mean diameter of inhibitory zone at different concentration using Kruskal Wallis Test.

Organisms	Concentrations	N	Mean Zone of inhibition in mm	SD	p
Streptococcus gordonii	15%	3	11.200	0.100	0.021
	25%	3	15.666	0.321	
	40%	3	16.000	0.264	
	50%	3	18.566	0.351	
Streptococcus salivarius	15%	3	0.000	0.000	0.013
	25%	3	0.000	0.000	
	40%	3	0.000	0.000	
	50%	3	16.000	0.200	
* p<0.05 was considered significant					

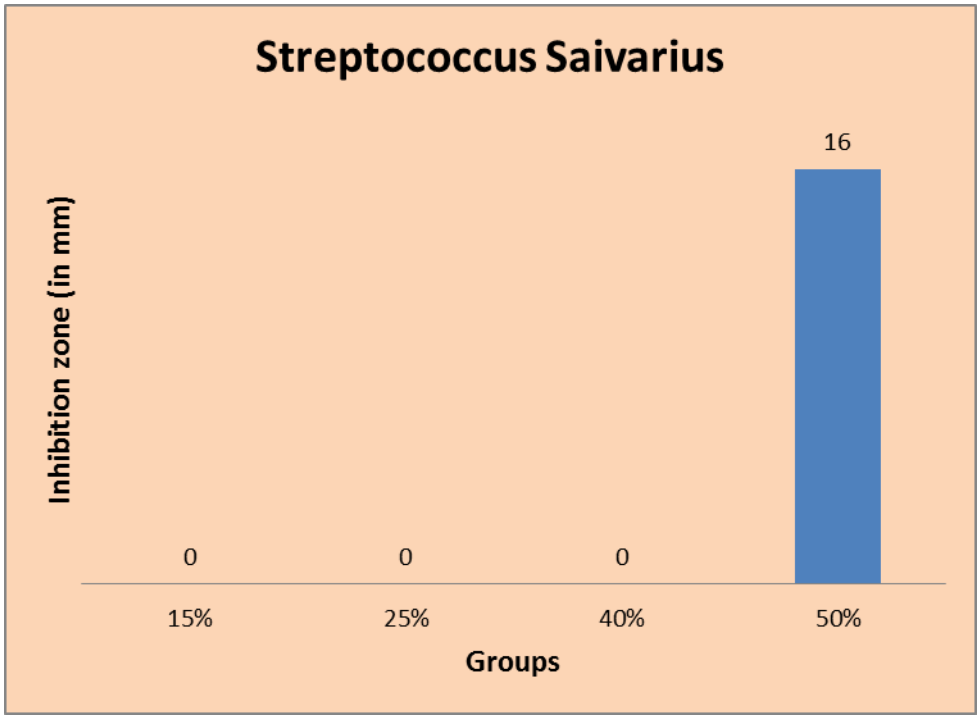
N= Number of samples, SD= Standard Deviation, p= Probability

Table 2: Pairwise comparison of mean diameter of inhibitory zone at different concentration between different groups.

Dependent Variable	Reference Group	Comparison Group	Mean Difference in mm	p
Streptococcus gordonii	15%	25%	-4.466	0.050
		40%	-4.800	0.050
		50%	-7.366	0.050
	25%	40%	-0.333	0.261
		50%	-2.900	0.050
	40%	50%	-2.566	0.050
Streptococcus salivarius	15%	25%	0.000	1.000
		40%	0.000	1.000
		50%	-16.000	0.037*
	25%	40%	0.000	1.000
		50%	-16.000	0.037*
	40%	50%	-16.000	0.037*
* p<0.05 was considered significant				



Graph 1: Graphical representation of Antibacterial activity of Ruta graveolens against S. gordonii.



Graph 2: Graphical representation of Antibacterial activity of Ruta graveolens against S. salivarius.

DISCUSSION

Emergence of multidrug resistantmicroorganisms is one of the major problems in antimicrobial therapy. So, there is a need for new antimicrobials from other sources, including plants. Medicinal plants are considered as potential sources of new chemotherapeutic drugs because of their diverse phytochemicals and little or no toxic effect.[12] In the present study, two of the primary colonizers of dental plaque, S. gordoniiand S. salivarius, were tested for their susceptibility to methanolic extract of R. graveolens. To the best of our knowledge this the first study reporting the antibacterialactivity of *R. graveolens* against *S. gordonii* and*S. salivarius*. The extractshowedfavourable antibacterial activity against the testedorganisms. The results illustrated that methanolic extract of R.graveolens showed the largest zone of inhibition about 18.6 mm against S.gordonii and 16 mm against S.salivarius at 50mg/ml. Average diameter of inhibitory zone is directly proportional to the concentration of plant extract against the bacterium S.gordonii. At concentrations, 15 mg/ml, 25 mg/mland 40 mg/ml, no antibacterialactivity of the plant extract was seen against S. salivarius. But antibacterial activity was observed at concentration of 50 mg/ml against S. salivarius. Antibacterial activity against S.gordonii at concentration of 50 mg/ml is higher than the S.salivarius at 50mg/ml. S. gordonii was observed to be more susceptible than S. salivarius at concentration 50 mg/ml. No statistically significant of different concentrations of

the R. graveolens extract among the same species (S.gordonii) was observed. But statistically significant of different concentrations of the R. graveolens extract among thesame species (S.salivarius) was observed.

The results of the present study are in accordance to a study conducted by Salman et. al., wherein it was concluded that methanolic extract of R. graveolens exert significant antibacterial activity against S. mutans and S. sobrinus, and alsoshowed that, as the concentration of the plant extract increased from 40 to 100 mg/ml, the susceptibility of the tested organism remained the same. This study also found out metaboliteskokusaginineand γ-fagarine through fragmentation pattern.[4] γ-fagarine and kokusaginine have various healthbenefits including antibacterial, antifungal, antioxidant, cytotoxicity and other biological activities.[13] In a study conducted by Taheriazam et.al., concluded that both hexanic stark and leaves extracts of R. graveolens had inhibitory and bactericidal effects on Klebsiella pneumonia.[14] Better effects of methanolic extract of Ruta graveolens in our study could be attributed to the presence of a large amount of substances such as volatile oils, flavonoids, coumarin and furoquinolone alkaloids.

CONCLUSION

Based on the findings of the present study, it could be concluded that methanolic extract of R. graveolens exerts significant antibacterial activity against S. gordonii and S. salivarius which are the primary colonizers of the dental plaque. This study provides scientific insight to further determine the antimicrobial principles and investigate other pharmacological properties of Ruta graveolens. Further studies could include larger sample size, bacterial species, and concentrations of Ruta graveolens above 50% andin vivo testing of this plant extract against these organisms.

On the basis of the present finding, it could be concluded that Ruta graveolens leaves possess the capabilities of being a good candidate in the search for a natural antimicrobial agent against infections and/or diseases caused by S. gordonii and S. salivarius.

In the future, the incorporation of such tested green products into mouthwash, irrigant, local drug delivery, chewing gum, toothpaste, and dental floss could pave the path in the way of controlling periodontal disease.

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