International Journal of Integrative sciences, Innovation and Technology
(A Peer Review E-3 Journal of S*cience* I*nnovation* T*echnology*)
Section A – Basic Sciences; Section B –Applied and Technological Sciences; Section C – Allied Sciences
Available online at www.ijiit.net

Research Article

ANTIBACTERIAL ACTIVITY OF HIBISCUS ROSA-SINENSIS AND ROSA DAMASCENA PETALS AGAINST DENTAL PATHOGEN

VICTORIA.J*, ARUNMOZHI.V
PG and Research Department of Microbiology, Sengamala Thayaar EducationalTrust Womens College, Mannargudi-614001, Tamil Nadu, India

ABSTRACT
This study was conducted to evaluate the antibacterial activity of *Hibiscus rosa-sinensis* and *Rosa damascene* petals against dental pathogen. Dental caries pathogen was isolated and identified based on cultural, morphological and biochemical characteristics. Extracts of *Hibiscus rosa-sinensis* and *Rosa damascena* against the dental isolates by well diffusion method. *Streptococcus mutans* is the most important bacterium in the formation of dental caries. Several antibiotics are available to treat oral infections but these have several undesirable side effects. Thus, there is a need for alternative prevention and treatment options that are safe, effective and economical. Hence the search for alternative products continues and natural phytochemicals isolated from plants used in traditional medicine are considered as good alternatives to synthatic chemicals. The methanolic and ethanolic extract of a petal of *Hibiscus rosa-sinensis* and *Rosa damascena* were tested for its antimicrobial activity against dental pathogen *Streptococcus mutans* in different concentration. In the present study it was found that the high concentration of 300 µl methanol extract of *Hibiscus rosa-sinensis* showed strong activity (27.33±1.632) against *Streptococcus mutans.* This study suggests that intake of petals of *Hibiscus rosa-sinensis* reduce the dental caries.

KEYWORDS: Antibacterial activity, Hibiscus rosa-sinensis , Rosa damascene, Well diffusion method

INTRODUCTION
Tooth is composed of a crown that protrudes above the gum and the root that is inserted into a long socket. A dense coating called enamel protects the outer surface of the crown. The enamel is an extremely hard, non-cellular material composed of tightly packed rods of calcium phosphate the cannot be replaced once the root has matured. A layer of cementum, which anchors the tooth to the periodontal membrane that lines the sockets, surrounds the root. The major portion of the tooth inside the crown and the root is composed of a highly calcified material called dentin. The core contains the pulp cavity, which has blood vessels and nerves. The places where the bone protrudes from the crown are covered by connective tissue and a mucous membrane called gingival or gum. Dental caries is a multifactorial human disease that has widely affected many populations all over the world. Dental caries, also known as tooth decay or a cavity, is an infection that causes demineralization of the hard tissues (enamel, dentin and cementum) and destruction of the organic matter of the tooth, usually by production of acid by hydrolysis of the food debris accumulated on the tooth surface. All caries occurs from acid demineralization that exceeds saliva and fluoride remineralization, and almost all acid demineralization occurs where food (containing carbohydrate like sugar) is left on teeth[2].

The oral cavity is the breeding ground to a wide range of gram positive and gram negative bacteria. This dynamic microflora changes with respect to age, hormonal status, diet and health status of an individual.(Aas *et al.*,2005*)*, has found more than 700 bacterial species from healthy oral cavity. Some of these bacteria show specificity as to individual subjects, others are specific to particular site as within the oral cavity[1]. Oral biofilms harbouring pathogenic bacteria are among the major virulence factors associated with dental diseases such as caries and periodontitis[17]. The mouth which is the entrance to the digestive system ,provides an environment that supports is large and varied microbial population .The teeth are unlike any other exterior surface of the body. This allows the accumulation of masses of

microorganism and their products. Accumulation of microbes and their product called dental plaque which an intimately involved in the formation of dental caries or tooth decay. The pathogenic oral bacteria,which cause the disease dental caries, convert source and other carbohydrates into lactic acid,which in turn attacks the tooth enamel.

Essentially, all oral bacteria possess surface molecules that foster some type of cell-to- cell interaction[19]. Only a few specialized organisms, primarily *streptococci* are able to adhere to oral surfaces such as the mucosa and tooth structure[23]. *Streptococci* can colonize the tooth surface and initiate plaque formation by their ability to synthesize extracellular polysaccharides from sucrose, using glucosyltransferase[10,13]. This sucrose dependent adherence and accumulation of cariogenic *Streptococci* is critical to the development of pathogenic plaque. *Streptococcus mutans* survives in an extremely diverse, high cell density biofilm on the tooth surface. These bacteria are strongly associated with caries formation[4,8,14,15].

Different organisms like *S.mutans ,S .gordonii, Actinomyces, Lactobacillus acidophilus, Bacteroides, Neisseria and Treponema* are involved in creating dental caries. But the most prevalent and important organisms are *Streptococcus mutans* and *Lactobacillus acidophilus. Streptococcus mutans* is a gram-positive organism that is the primary causative agent in the formation of dental cavities in humans. *S.mutans*, a member of the human oral flora, is widely recognized as the main etiological agent of dental caries.*Streptococcus mutans* is the most common cariogenic bacteria associated with dental carries. Today it is believed to be the chief etiologic agent in human dental caries. This bacterium has the ability to metabolize dietary sucrose and synthesize glucan by cell surface and extracellular glucosyltransferase. This glucan is an insoluble sticky or slimy gel relatively inert and resistant to bacterial hydrolytic enzymes which causes plaque to adhere tenaciously to tooth surfaces[21].

The wide use of antibiotics in the treatment of bacterial infections has led to the emergence and spread of resistant strains. Thus, it is extremely important to find new antimicrobial agents or new ways that are effective for the treatment of infectious to diseases caused by drug-resistant bacteria.. Natural ayurvedic treatment will be more effective than antibiotics

Hibiscus rosa-sinensis (Family Malvaceae) is a shrub and grown as an ornamental plant. It is being used as analgesic anti-inflammatory, to treat trauma throughout Caribbean. Leaves are anodyne, emollient and aperients. It acts as antispasmodic for uterine and intestinal spasms. The flowers considered as emollient and demulcent. Flowers and leaf extracts (1%) in paraffin is used as hair growth promoting medicine (Anonymous, 1994; Khory et al., 1999).It is also being used in treatment of boils, burns, fever, cystitis, gonorrhea, a bronchial catarrh, headache, menstrual irregularities, prostate disorders, diarrhea, cough, asthma and toothache etc[3,11].

The rose petals have been reported to posses astringent property in traditional system of medicine; however, they have not been evaluated systemically for antimicrobial activity. Rose flowers have various medicinal uses such as eye tonic, female reproductive tonic. They have been recommended to be useful in various urogenital tract infections, fever and cough (The Ayurveda Encyclopedia, 1978)[22].

MATERIALS AND METHODS

Sample Collection

Dental swabs were collected from the infected persons with dental caries. The sterile cotton swabs were used to collect the sample from the surface of the teeth. The collected dental swab was immediately brought to the laboratory for the isolation of bacteria.

Isolation of Bacteria[9]

The dental plaque sample was inoculated into nutrient broth and incubated for 18-24 hours. Inoculated samples were streaked on nutrient agar and to the selective media. The pathogens were isolated and identified by Bergey's manual of systematic Bacteriology.

Identificaion of Bacteria[7,16]

The isolated bacteria was identified by Gram's staining, motility, and biochemical tests.

Phytochemical Analysis[20]

Detection of Carbohydrates

The extract (100 mg) is dissolved in 5ml of water and filtrate. The filtrate is subjected to the following tests.

Fehling's test

1 ml of filtrate is boiled on water bath with 1ml each of fehling's solution A and B.A red precipitate indicates the presence of sugar.

Fehling's solution A:

Copper sulphate (34.66g) is dissolved in distilled water and made up to 500 ml using distilled water.

Fehling's solution B:
Potassium sodium tartarate (17.3g) and sodium hydroxide (50g) is dissolved in water and made up to 500ml.

Detection of Glycosides
50mg of extract is hydrolysed with concentrated hydrochloric acid for 2 hour on a water bath, filtered and the hydrolysate is subjected to the following tests.

Borntrager's test
To 2ml of filtered hydrolysate,3 ml of chloroform is added and shaken chliroform layer is separated and 10% ammonia solution is added to it. Pink colour indicates the presence of glycosides.

Detection of Protein and Amino acid
The extract (100mg) is dissolved in 10ml of distilled water and filtered through whatmann No.1 filter paper and filtrate is subjected to tests for protein and amino acid.

Ninhydrin test
2 drops of ninhydrin solution (10mg of ninhydrin solution in 200 ml of acetone) are added to 2 ml of aqueous filtrate. A characterstics purple colour indicates the presence of amino acid.

Detection of Alkaloids
Solvent free extract,50 mg is stirred with few ml of dilute hydrochloric acid and filtered. The filtrate is tested carefully with various alkaloidal reagent as follows:

Mayer's test
To a few ml of filtrate, a drop or two of mayer's reagent are added by the side of the test tube .A white or creamy precipitate indicates the test as positive.

Detection of Saponins
The extract (50 mg) is diluted with distilled water and made up to 20 ml. The suspension is shaken in a graduated cylinder for 15 min. A two cm days of foum indicuted the presence of saponins.

Detection of Flavonoids
2 ml filtrate was added to concentration HCL and magnesium ribbon. Pink tomato red colour indicated the presence of Flavonoids.

Detection of Tannins
1 ml of extract was treated with few drops of 0.1% ferric chloride and observed for browinsh green or blue-black coloration.

Preparation of flower extracts
Petals of *Hibiscus rosa-sinensis* and Rosa *damascena* were extracted with three different solvents such as ethanol, methanol and distilled water. 20 gm of flower powder was extracted in 100 ml of each solvent. Solvent extract was then filtered through whatmann filter paper no. 1. These extracts were kept for evaporation under reduced pressure to yield residue. This residue was collected and different concentrations of extracts were prepared using respective solvent.

Inoculum Preparation
The bacteria were inoculated into liquid medium (Nutrient broth) and incubated at $37^{O}C$ for 4 hrs and suspensions were checked to provide approximately 10 CFU/ml.

Antibacterial Activity (Well diffusion Method)[12]
20 ml of Muller Hinton agar was prepared and cooled at $45^{O}C$ and was poured into Sterile petri plates and allowed to solidify completely. A lawn of test pathogen was prepared by evenly spreading 0.1 ml inoculum with the help of a sterilized spreader onto the entire surface of agar plate. Antibacterial activity of extracts was evaluated by agar well diffusion method. After the medium was solidified, wells of 6mm were made in the plates with the help of a cork borer. 200 µl of the extracts (500 mg /1ml) was introduced into the wells separately and the plates were incubated overnight at $37^{o}C$. The experiment was performed under strict aseptic conditions. Plates were incubated at $37^{o}C$ for 24 hrs and diameters of inhibition zones were determined.

Statistical Analysis[6]
The results obtained in the present investigation were subjected to statistical analysis like mean (X) and standard deviation (SD).

RESULTS AND DISCUSSION
In the present study antibacterial properties of petals of *Hibiscus rosa-sinensis* and *Rosa damascena* was explored against dental pathogen .Antibacterial components were extracted by using different solvents such as ethanol and methanol. The antibacterial nature of extracts were assessed by agar well diffusion method.

Table 1: Morphological and Biochemical characteristics of *Streptococcus mutans*

S.No	Name of the test	*Streptococcus mutans*
1	Gram'staining	+
2	Motility	Non-Motile
3	Shape	Cocci
4	Indole test	-
5	Methyl red test	-
6	Voges-Proskauer test	+
7	Citrate utilization test	-
8	Catalase	-
9	Urease test	-

(+) Positive, (-) Negative

Isolation and Identification of Bacteria

The bacterial colonies were isolated from dental swab and it was identified as *Streptococcus mutans* by Gram staining and biochemical characteristics. The identified bacteria was transferred to fresh nutrient ager plate and the pure culture was maintained for the isolated pathogen *Streptococcus mutans.*

Characteristics of dental isolate

It was gram positive cocci. The cells were arranged in pairs or chains. They are catalase negative. They are fermentative. (Table-1) and confirmed as *Streptococcus mutans* using Bergey's manual of Systematic Bacteriology.

Phytochemical Analysis

The phytochemical analysis of flower petals of (hibiscus rosa-sinensis and Rosa damascene) showed the presence of many biologicaly active constituents as depicted in (Table-2).Alkaloids, carbohydrates & glycosides, saponins, protein and flavonoids were detected in both Hibiscus rosa-sinensis,Rosa damascene.

Table 2: Qualitative analysis of dried Hibiscus petals

S.No	Phytoconstituents checked	Dried Hibiscus petals	Dried rose petals
1	Alkaloids	+	-
2	Carbohydrates and glycosides	+	+
3	Saponins	+	+
4	Tannins	+	+
5	Proteins /Amino acids	+	-
6	Flavonoids	+	+

Antibacterial activity of petals extracts against dental isolates

In order to check the antimicrobial activity of extracted petal samples (*Hibiscus rosa-sinensis* and *Rosa damascena*),agar well diffusion method of Kirby Bauer was used (Table-3) showed the results of zone of inhibitions observed for antibacterial activity.

The antibacterial efficiency of *Hibiscus rosa-sinensis* and *Rosa damascene* were tested against the dental isolate *Streptococcus mutans* and it was quantitatively assessed by the presence or absence of zone of inhibition and it was measured in diameter using the zone scale.

Table 3: Antibacterial activity of Hibiscus rosa-Sinensis against *Streptococcus mutans*

S.No	Solvents	Zone of inhibition (mm)		
		100 µl	200 µl	300 µl
1	Ethanol	15.33 ±1.246	21.33 ±2.494	23.33 ±3.943
2	Methanol	16.66 ±1.2247	21 ±2.160	27.33 ±2.494
3	Water	-	-	-

Table 4: Antibacterial activity of Rosa damascena against *Streptococcus mutans*

S.No	Solvents	Zone of Inhibition (mm)		
		100 µl	200 µl	300 µl
1	Ethanol	15 ±0.942	20.66 ±1.246	23 ±1.632
2	Methanol	18 ±1.247	23.66 ±1.256	25 ±1.621
3	Water	-	-	-

Effect of *Hibiscus rosa sinensis* on *Streptococcus mutans*

The ethanolic and methanolic exctract of *Hibiscus rosa sinensis* were used against dental pathogen, *S.mutans* in different concentration such as 100 µl, 200 µl and 300 µl.

On *Streptococcus mutans*, 300µl of ethanolic extracts of *Hibiscus rosa-sinensis* showed the highest activity (23.33±3.943) followed by 200µl extract (21.33±2.494).100µl extracts (15.33+1.246) showed lowest activity against dental pathogen.

In the same way, on *streptococcus mutans*, 300µl of methanolic extract of *Hibiscus rosa-sinensis* showed the maximum activity (27.33±2.494) followed by 200µl (21±2.160) and 100µl (16.66±1.2247)

Effect of *Rosa damascena* on *Streptococcus mutans*

The ethanolic and methanolic extract of *Rosa damascena* was used against oral pathogen, *Streptococcus mutans* in different concentration such as 100 µl, 200 µl and 300µl.

On *Streptococcus mutans*,300µl of ethanolic extract of *Rosa damascena* showed the maximum activity (23±1.632) followed by 200µl of extracts (20.66±1.246) and 100µl extract showed minimum activity (15±0.942).

On *Streptococcus mutans*, 300µl of methanolic extract of *Rosa damascena* showed the maximum activity (25 ±1.632) followed by 200µl of extract (23.66±1.246) and 100µl extract showed the moderate activity (18±1.247).

Among the two extracts (ethanol and methanol) of petals, the methanolic extract of *Hibiscus rosa-sinensis* showed highest activity against dental pathogen, *Streptococcus mutans.* Aqueous extracts does not showed any effect on *Streptococcus mutans.*

CONCLUSION

In the present study, dental caries pathogen were isolated from infected persons. The dental caries pathogen were identified based on cultural, morphological and biochemical characteristics. Extracts of *Hibiscus rosa-Sinensis* and *Rosa damascena* against the dental isolates were assessed by well diffusion method. *Streptococcus mutans* is the most important bacterium in the formation of dental caries. Several antibiotics are available to treat oral infections but these have several undesirable side effects. Thus, there is a need for alternative prevention and treatment options that are safe, effective and economical. Hence the search for alternative products continues and natural phytochemicals isolated from plants used in traditional medicine are considered as good alternatives to synthetic chemicals. The methanolic and ethanolic extract of flower petals of *Hibiscus rosa-sinensis* and *Rosa damascena* were tested for its antimicrobial activity against dental pathogen *Streptococcus mutans* in different concentrations. In the present study it was found that the high concentration of methanolic extract of *Hibiscus rosa-sinensis* showed strong activity against *Streptococcus mutans.* This study suggests that intake of petals of *Hibiscus rosa-sinensis* reduce the dental caries. The petal is low cost, easily available and no side effect.

ACKNOWLEDGMENTS

The authors are thankful to Dr.V.Divaharan, Correspondent, S.T.E.T Womens college, for his keen interest and constant encouragement

REFERENCES

1. *Aas JA, Paster BJ,Stokes LN,Olsen I.,2005. Defining the normal bacteria flora of the oral cavity. J.clin.microbial.vol.43, pp.5721-5732.*
2. *Adwan MG, Abu-Shanab AB and Adwan MK.,2008.In vitro activity of certain drugs in combination with plant extracts against Staphylococcus aureus infections. Pakistan Journal of Medicine.vol.24,pp. 541-544.*
3. *Anonymous. 1994. The Useful Plants of India. Publication and Information Directorate, CSIR, New Delhi.*
4. *Bauer,A.W.,Kirby.,1986.Antibacterial susceptibility testing by standard single disc diffusion method. American journal of clinical pathology.vol.45,pp.493-496.*
5. *Burne R A, Chen YY and Penders JE.,1997. Analysis of gene expression in Streptococcus mutans in biofilms in vitro. Adv Dent Res.vol.11,pp. 100-109.*
6. *Gupta,S.P.1977.Statistical method.S.Chands and co.New Delhi.*

7. *Han's Christian Gram.,1884.cellular responses of B.subtilis and E.coli to the gram stain. J. of Bacterialogy. vol.156 ,pp.846-858.*
8. *Hamilton I R.,2000. Ecological basis for dental caries. In Oral Bacterial Ecology: the Molecular Basis,pp.219-264. Edited by H. K.Kuramitsu & R.P.Ellen. Wymondham, UK: Horizon Scientific Press.*
9. *Holt JG, Kreig NR, Sneath PHA, Stanely JT and Willams ST.,1994. In (eds.) Bergey's Manual of Determinative Bacteriology. 9th ed Lippincott, Willams and Wilkins, Baltimore.*
10. *Jacquelin LF, Brisset L, Lemagrex E, Carquin J, Gelle MP, Choisy C.,1995. Prevention of cariogenic dental plaque. Study of the structure implicated in the Streptococcus mutans and Streptococcus sobrinus adhesion and coaggregation.Pathol.Biol.vol.43,pp.371-379.*
11. *Khory, R.N.,Katrak,N.N.,1990.Materia Medica of india and Therapeutics. Komal Prakashan , New Delhi.*
12. *Kirby Bauer.,1960. Antibiotic Susceptibility of testing by a standard single disc method. Journal Clinical Pathology, vol.36, pp.492.*
13. *Koo H, Gomes BPFA, Rosalen PL, Ambrosano GMB, Park YK, Cury JA.,2000. In vitro antimicrobial activity of propolis and Arnica Montana against oral pathogens. Arch. Oral. Biol.vol. 45(2),pp.141-148.*
14. *Kolenbrander PE.,2000.Oral microbial communities: biofilms, interactions , and genetic systems. Annu Rev Microbial.vol.54,pp.413-437.*
15. *Liljemark WF and Bloomquist C.,1996. Human oral microbial ecology and dental caries and periodontal diseases.Crit Rev Oral Biol Med .vol.7 , pp.180-198.*
16. *Macfaddin,J.F.,1989. Biochemical tests for identification of medical bacteria. Williams and Wilkins, Baltimore.*
17. *Marsh PD.,2003. Are dental diseases examples of ecological catastrophes. Microbiology.vol.149,pp.279-294.*
18. *Munson MA, Banerjee A, Watson TF and Wade WG.,2004. Molecular analysis of the microflora associated with dental caries. J Clin Microbial.vol.42,pp.3023-3029.*
19. *Newman MG, Takei HH, Klokkevold P, Carrenza FA.,2006 Carranza's Clinical Periodentol.vol.10(9),pp.132-142.*
20. *Raaman. N., 2006.Phytochemical technique ISBN,pp. 81-89.*
21. *Shafer, Hine, Levy A., 2001.Textbook of Oral Pathology. Fourth ed, Saunders, Pennsylvania.*
22. *The Ayurveda Encyclopedia., 1978. Swami Sadashiva Thrita, Sir Satguru* Publications. A division of Indian Book Center, Delhi 11007.
23. *Theodore MR, Harald OH, Edward JS.,2006.Strurdevanat's Art and Science of Operative Dentistry.5th Ed.pp. 75-93.*
24. *Wilson M., 2001. Bacterial bioflims and humans disease. Sci Prog.vol.84,pp.235-254.*