Research Article

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

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ABSTRACT
This study was conducted to evaluate the antibacterial activity of Hibiscus rosa-sinensis and Rosa damascena 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 synthetic 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. 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...
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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. Only a few specialized organisms, primarily streptococci, are able to adhere to oral surfaces such as the mucosa and tooth structure. Streptococci can colonize the tooth surface and initiate plaque formation by their ability to synthesize extracellular polysaccharides from sucrose, using glucosyltransferase. 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.

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 carries. 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.

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 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.

The rose petals have been reported to possess 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).

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
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.

Identification of Bacteria
The isolated bacteria was identified by Gram’s staining, motility, and biochemical tests.

Phytochemical Analysis
Detection of Carbohydrates
The extract (100 mg) is dissolved in 5 ml 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 1 ml 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 tartrate (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 chloroform 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 characteristics 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 foam indicated 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 brownish 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°C for 4 hrs and suspensions were checked to provide approximately 10 CFU/ml.

Antibacterial Activity (Well diffusion Method)
20 ml of Muller Hinton agar was prepared and cooled at 45°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°C. The experiment was performed under strict aseptic conditions. Plates were incubated at 37°C for 24 hrs and diameters of inhibition zones were determined.

Statistical Analysis
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.
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 agar 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 biologically active constituents as depicted in (Table-2). Alkaloids, carbohydrates & glycosides, saponins, protein and flavonoids were detected in both Hibiscus rosa-sinensis, Rosa damascene.

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

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

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

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

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

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**REFERENCES**