

Antibacterial Effect of Abelmoschus Esculentus (Okra) Extracts on Dental Caries Derived Streptococcus Mutans.

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Abstract

Introduction: *Streptococcus mutans* (S.mutans) have the ability to survive in an acid environment by modulating sugar metabolic pathways coupled with irreversible binding to teeth which is a key component to its pathogenesis in dental caries. Usage of traditional plants and natural products for the treatment of infections rather than synthetically derived drugs are on a steep rise. The plant of interest in the present study is *Abelmoschus esculentus* (okra).

Objectives : To access the antibacterial effect of extracts of various parts of the okra vegetable on *S. mutans*.

Materials and Methods: Strains of *S. mutans* obtained from caries lesions of patients were subcultured using brain heart infusion agar and antibacterial effect of the okra extracts were determined using agar well diffusion technique.

Results and Discussion: The highest zone of inhibition was recorded (4mm) when the combination of peel and seed was used. It was also noted that all the combinations of the plant extract produced antibacterial activity that was at least equal to or higher than the activity of the positive control (Ofloxacin).

Conclusion: Okra extracts can be used as a natural antibacterial agent against cariogenic bacteria like *S.mutans*.

Keywords: *Abelmoschus esculentus*, okra, anticariogenic agent ,antibacterial effect, *Streptococcus mutans*.

I. Introduction

The oral microflora is a complex ecosystem which contains a wide variety of bacterial species. *Streptococcus mutans* (S.mutans) was isolated from human carious lesions by Clake(1) in 1924. The establishment of *S. mutans* in the oral cavity of human beings is always being emphasized because they are the principal bacteria responsible for dental caries (2). *S. mutans* are major cariogenic organisms by the virtue of their ability to produce large quantities of glucans as well as acids, exceeding the salivary buffering capacities. This unique characteristic gives the bacteria an advantage to outcompete noncariogenic commensal bacterial species at low pH environments. The ability to survive in an acid environment by modulating sugar metabolic pathways coupled with irreversible binding to teeth is a key component to *S. mutans* pathogenesis (3).

There have been numerous reports of the use of traditional plants and natural products for the treatment of oral diseases. Many plant-derived medicines used in traditional medicinal systems have been recorded in pharmacopoeias as agents used to treat infections and a number of these have been recently investigated for their efficacy against oral microbial pathogens. The general antimicrobial activities of medicinal plants and plant products, such as essential oils, have been reviewed previously (4).

Okra (*Abelmoschus esculentus*) or bhendi also known as ladies finger is an important vegetable crop being native of tropical Africa. It is a tall annual dicotyledonous plant related to cotton (5). Okra mucilage has been used for several medicinal applications (6). However to date the effect of okra on the *S. Mutans* has not been studied. Thus this study was conducted with the objective of accessing the effect of extracts of various parts of the okra vegetable on *S. Mutans*.

II. Materials And Methods

The present study was conducted by the Department of Pedodontics and Preventive Dentistry, Rajah Muthiah Dental College and hospital, Annamalai University in collaboration with Department of Microbiology, Rajah Muthiah Medical College and hospital, Annamalai University. ethical clearance was obtained from the

institutional ethical committee keeping in mind Helsinki Declaration of 1975 that was revised in 2000. A qualified medical microbiologist supervised the study. Various combinations of peel and seeds of *Abelmoschus esculentus* were used in the study with the use of either ethanol or methanol as solvents for the extracts.

Extract preparation

One kilogram of fresh okra vegetable was obtained from the local vegetable markets. The vegetables were inspected carefully and the diseased vegetables were discarded. The selected vegetables were then thoroughly washed with sterile distilled water to remove any dust and contaminants. Then from each vegetable the peel and seeds were separated. The separated vegetable parts were then air dried under sunshade for 5 days. The dried parts were then ground separately to a fine powder in an electrical mixer. 5 grams each of the dried plant powder were weighed and placed in two separate test tubes containing 25 ml acetone and 25 ml ethanol respectively. The mixture was then allowed to stand at room temperature (37°C). The preparation was stirred once every 24 hours and at the end of 72 hours the extracts were filtered through Whatman's filter paper no 1 and the filtered contents were further allowed to dry for another 24 hours. The final preparation thus obtained was again mixed with 2 ml of either solvents and this mixture was used for the antimicrobial assay.

Antimicrobial assay

Strains of *S. mutans* isolated from carious lesions of 10 patients were subcultured on Brain Heart Infusion Agar (BHIA) (Himedia company Mumbai) and after 24 hours the fresh colonies were obtained. Bacterial suspension was prepared by mixing the fresh colonies with sterile distilled water and this was adjusted to McFarland opacity no 0.5 and the standardised preparation obtained was used for the assay. Determination of antibacterial activity in the present study was done using the Agar well diffusion method. BHIA was prepared and poured into sterile petriplates. After solidification 4mm wells were cut with a sterile microtip and about 50 ml of the test agents were placed into the wells. Commercially available Ofloxacin was used as a positive control. The plates were incubated at 37°C for 24 hours and the zone of inhibition (the measure for antibacterial activity) was recorded (Fig.1).

III. Results

The highest zone of inhibition was recorded (4mm) when the combination of peel and seed was used. Similar level of activity was observed when either ether or acetone was used as the solvent. However better antibacterial activity was seen when skin was used with ether and peel was used with acetone (3mm in both the samples). It was noted that all the combinations of the plant used in the study with either acetone or ether as solvents produced antibacterial activity that was higher than the activity of the positive control (Ofloxacin)(Table :1, Fig: 2).

On statistical analysis it was found the mean zone of inhibition was lowest for ofloxacin(control) which was (mean=1.80,SD=0.789). Among the extracts lowest zone of inhibition was observed in peel ethanolic extract (mean=2.00,SD=.667) and the highest zone of inhibition was observed in seed acetonetic extract (mean=3.2,SD=.789)(Table:2).

Peel and seed ethanolic as well acetonetic extracts have zone of inhibitions(mean=3.1,SD=.738) and (mean=3,SD=.943) respectively.

IV. Discussion

The global need for alternative prevention and treatment options and products for oral diseases that are safe, effective and economical comes from the rise in disease incidence (particularly in developing countries), increased resistance by pathogenic bacteria to currently used antibiotics and chemotherapeutics, opportunistic infections in immunocompromised individuals and financial considerations in developing countries.(7,8) Despite several chemical agents being commercially available, these can alter oral micro biota and have undesirable side-effects such as vomiting, diarrhea and tooth staining. (9, 10) 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 (11).

Okra (*Abelmoschus* spp.) is a traditional vegetable crop with considerable area under cultivation in Africa and Asia. It has been called "a perfect villager's vegetable" because of its robust nature, dietary fibers and distinct seed protein balanced in both lysine and tryptophan amino acids (unlike the proteins of cereals and pulses) it provides (6). However to date there have been no studies reported about the effect of okra on *Streptococcus mutans*. Hence the present in-vitro study was designed to evaluate the antibacterial activity of various parts of the okra vegetable on the *S. Mutans* microorganism.

In the present study the highest mean zone of inhibition was reported against *S. Mutans* when seed acetonetic extract was used. (Table: 3, Fig: 3)

Pods and seeds are rich in phenolic compounds with important biological properties like quaternary derivatives, catechin oligomers and hydroxycinnamic derivatives (12).

Phenolic compounds are known to act by the alteration of the permeability of the bacterial cell membrane that could result in the uncoupling of oxidative phosphorylation, inhibition of active transport, and loss of pool metabolites from bacteria (13). This could be the possible explanation to the antibacterial activity of okra extracts reported in the present study.

In the present study, the zone of inhibition of ofloxacin was comparatively less as compared to various extracts. This could be possibly due to development of antibiotic resistance to certain drugs by *S. mutans*.

Okra is a highly nutritious vegetable that is non-toxic and safe for consumption even for young children and pregnant women. However, its safety profile as a clinical medicament is yet to be established. Furthermore, a crude extract was used in the present study. Thus, further studies are recommended to determine the minimal inhibitory concentration of the extracts on *Streptococcus mutans* as well as to explore its inhibitory effects on other common pathogenic microorganisms of the oral cavity.

V. Conclusion

Okra extracts can be used as a natural antibacterial agent against cariogenic bacteria like *S. mutans*. The antibacterial effect of okra is markedly superior when compared to ofloxacin. Okra extracts could be used as an anticariogenic agent after conducting clinical trials to ascertain its safety as well as antimicrobial and antibacterial effects on pathogenic and commensals inhabiting the oral cavity.

Conflict of interest: NIL

Funding: NIL

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Figure Captions

Fig.1 Culture plates showing zone of inhibition

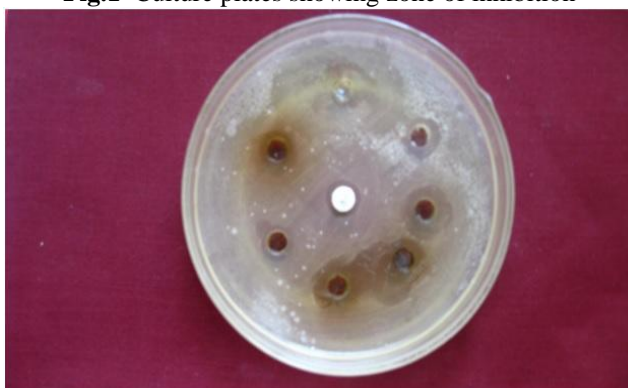


Fig.2 Zone of inhibition for acetonic extract and ofloxacin (control)

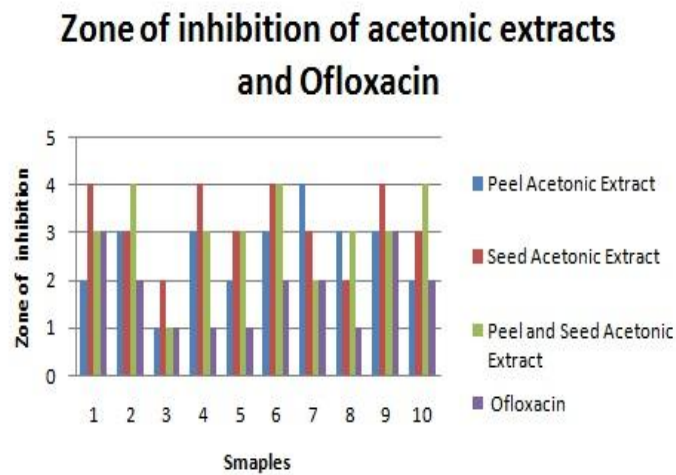


Fig.3 Mean and Standard deviation of zone of inhibitions

