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Assessment of Antimicrobial Activity of Carica Papaya Seed Extract on Porphyromonas Gingivalis, Aggregatibacter Actinomycetemcomitans and Prevotella Intermedia: An in-Vitro Study

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Abstract

Aim & objective: Assessment of antimicrobial activity of carica papaya seed extract on Porphyromonas gingivalis (Pg), Aggregatibacter actinomycetemcomitans (Aa) and Prevotella intermedia (Pi) : an in-vitro study

Materials and methods: Aqueous and ethanolic extracts of Carica papaya seed was prepared. Ethanolic extract has shown better results than aqueous extract hence ethanolic extract was obtained in 1%, 2.5% and 5% concentrations. The antimicrobial tests were conducted they are disc diffusion assay, minimum inhibitory concentration, minimum bactericidal concentration. Chlorhexidine and normal saline was used as the positive and negative control respectively.

Results: In Disc diffusion assay, ethanolic extract has shown better results against Pi>Aa>Pg, MIC has shown better results against Aa>Pg>Pi MBC has shown better results against Aa>Pi>Pg.

Conclusion: The ethonolic extract has shown better results than aqueous extract of Carica papaya seed, If there was no growth of bacteria it was considered as BACTERICIDAL effect, if there was growth of bacteria then BACTERIOSTATIC effect was considered.

Keywords: Aggregatibacter actinomycetemcomitans (Aa) carica papaya, carica papaya seed, Porphyromonas gingivalis (Pg), and Prevotella intermedia (Pi)

Introduction

Periodontitis is a chronic inflammatory disease triggered by bacteria that progressively damage the connective tissues and alveolar bones and is the most common cause of tooth loss experienced by adults. Periodontitis is considered to be epidemic disease with highest prevalence worldwide. According to studies it is said that almost 40-90% of global population suffers from periodontal disease.[1] Evidences tell that use of artificial antimicrobial and antibiotic causes anti-microbial resistances. Natural phytochemicals have demonstrated to be worthy substitutes to synthetic agents.[2]

Studies found that in adults with chronic periodontitis, both non-surgical and surgical therapy are used for the treatment. Along with scaling and root planning use of an adjunctive antimicrobial mediator increases patient outcomes over a period of time compared to scaling alone. Various antimicrobials and chemotherapeutic agents have been tried and tested in the management of periodontal disease.

Herbal remedies have been sought to achieve antimicrobial, anti oxidant, antiseptic, antiflammatory effects. The alternatives to the antibiotic as a periodontitis therapy is constantly developed, one of them is the herbal materials from papaya.

Carica papaya is a neutraceutical plant having a wide range of pharmacological activities.[3] Carica papaya belongs to the family of Caricaceae, and several species of Caricaceae have been used as remedy against a variety of disease. The fruit juice and leaf extract have been demonstrated to have a wide variety of properties including anticancer, antioxidative, anti-inflammatory, anti-bacterial, nephroprotective, hepatoprotective, hypoglycemic and hypolipidemic effects, and anti-sickling effect in sickle cell disease. The leaf extract has also been shown to have larvicidal properties against the Aedes aegypti mosquito, the vector of the DENV.[4]

Papaya seeds possess proteins, fatty acids and phospholipids, such as phosphotidylcholine and cardiolipin. The well studied proteinases from papaya are papain, chymopapain, caricain, and glycylendopeptidase.[3]

To date, the influence of papaya seed extract to on periodontal pathogen causing periodontitis has not been explored, therefore, the present research aims to assess the antimicrobial activity of Papaya (Carica papaya) seed extract on the periodontal pathogens-

(a) Porphyromonas gingivalis (Pg)

(b) Aggregatibacter actinomycetemcomitans (Aa) and

(c) Prevotella intermedia (Pi)

Materials and methods

This study was conducted at Maratha Mandal's Nathajirao G. Halgekar Institute of Dental Science & Research Centre, Belagavi in the Department of Central Research of Laboratory

Extract Preparation

Preparation of Aqueous and Ethanol Seed extract

Aqueous Extract

Seeds of carica papaya was collected from the ripened fruits and washed thoroughly with distilled water for 3times. The seeds were allowed to dry at room temperature. The dried seeds were powered using a grinder. 30gms of dried seeds powder were mixed with 300ml of distilled water and heated at 70degree for 20mins. The extract was filtered using cheese cloth followed by whattman no. filter paper. The extract was then concentrated using rota-vapour at 75- 80 degree Celsius under vacuum for 3-4 hours. The concentrated extract was finally dried using freeze drier to obtain

crude papaya seed extract, which was stored at 4degree Celsius.

Ethanolic Extract

The 30gms of dried seeds of carica papaya were mixed with 300ml of ethanol and allowed to soak for 2days with occasional shaking. The extract was filtered with ordinary filter paper followed by whattman filter no. 1 paper. The extract was then concentrated using rota-vapour under vacuum at 65degree Celsius for 1hour. The concentrated extract was air dried using water bath at 50degree Celsius. The extract was transferred into sterile container and stored at 4degree Celsius.

Microbial Assessment

An in vitro microbiological study has been carried out to test papaya seed extract on standard strains of

A. actinomycetncomitans, P.gingivalis and P. intermedia in the lab.

Disc Diffusion Assay

Blood agar was used. Agar plates were brought to room temperature before use. Using a loop or swab, colonies were transferred to the agar plates. Entire surface of agar plate was swabbed three times, rotating plates approximately 60º between streaking to ensure even distribution. Inoculated plate was allowed to stand for at least 3 minutes before making wells. Hollow tube of 5mm heated and pressed on the plates to make 5 wells which were added with 75µl, 50 µl, 25 µl, 10 µl and 5 µl of the extract and inoculated for 48-72 hours. Diameter of inhibition zone was measured to nearest whole millimeter by holding the measuring device.

Minimum Inhibitory Concentration (MIC)

9 dilutions of each drug done with Thioglycollate broth. Initial tube 100µL of drug added into the 300µL of Thioglycollate broth. 200µL broth in rest 9 tubes. Then from the initial tube 200µL transferred to the first tube containing 200µL of broth. The serial dilution was repeated in 2 fold dilution for each drug. MIC values were determined as the lowest concentrations, at which no bacterial growth occurred.

Minimum Bactericidal Concentration (MBC)

Culture plating was done using the tubes showing sensitivity to the extract in MIC. Incubated for a period of 24-48 hours in anaerobic CO_2 jar followed by colony count.

- If there was no growth -BACTERICIDAL effect
- If there was growth - BACTERIOSTATIC effect

Results

Disc Diffusion Assay

	Pg	Pi	Aa
Ethanol extract	12.25mm	10.05mm	12.05mm
Aqueous extract	10.05mm	11.33mm	12.05mm
Chlorhexidine	16.5mm	17.5mm	16.05mm

Minimum Inhibitory Concentration & Minimum Bactericidal Concentration of aqueous extract

	Porphyromonas gingivalis	Prevotella intermedia	Aggregatibacter Actinomycetem-comitans
MIC	16.53±0	18.53±0	15.53±0
MBC	78.12	48.34	34.56

Discussion

To the best of our knowledge, this in-vitro study is the first of its kind using the natural seed extracts of C. papaya plant in both the aqueous and ethanolic variants on periodontal pathogens namely Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans and Prevotella intermedia. Comparative results between the two suggest that the ethanol seed extract has a superior bactericidal action as compared to the aqeous seed extract against these periodontal pathogens. The results also suggest the similar action of ethanol seed extract against Prevotella intermedia and Aggregatibacter actinomycetemcomitans as compared to chlorhexidine. However, the later proves to be superior against Porphyromonas gingivalis in its actions. The reason for the superiority of the ethanol extract cannot be attributed at the present and demands further studies to be conducted in the future. This study is in relation to a study conducted by Somanah J et al (2013) using Fermented Papaya Preparation (FPP), to check its efficacy on growth, hydrophobicity and acid production of Streptococcus mutans, Streptococcus mitis and Lactobacillus acidophilus. An overall oral health evaluation was done using FPP in diabetic patients. FPP has shown significant reduction in microbial load when tested in vitro. However, the FPP used in this study was derived commercially unlike the present study and tested on different micro-organisms.

This study is in relation to a study conducted by Faisal et al, using papaya seed and papaya leaf extract against S. aureus, P. aeruginosa and E. coli with increasing seed extract concentration as 25, 50, 75 and 100mg/ml, zone of inhibition was not found at 25,50,75mg/ml but at 100mg/ml 2.5mm zone of inhibition was seen.

Conclusion

Hence, to conclude, in the present day scenario when resistance to drugs is gradually increasing, finding an alternative in herbal medicine is gaining wide popularity. An alternative in natural papaya products also reduces the risk of side effects and proves to be economical.

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