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# CARICA PAPAYA: A FRUITFUL ALTERNATIVE AGAINST PERIODONTOPATHOGENS ??

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## ABSTRACT

Papaya (*carica papaya* linn.) contains broad spectrum phytochemicals of nutritional and medicinal importance like polysaccharides, vitamins, minerals, enzymes, proteins, alkaloids, glycosides, lectins, saponins etc. which may be accountable for its antimicrobial property. So the objective of this study is to assess and study antimicrobial efficacy of phytochemicals extracted from papaya fruit and leaf on periodontal pathogen. Aqueous and ethanolic extracts of unripe papaya fruit and leaves are made respectively and the extracts have been tested for their antimicrobial activity on the cultures of various periodontal pathogens in vitro by well dilution method. and the results are, the MIC for aggregatibacter actinomycetemcomitans with ethanolic fruit extract, aqueous fruit extract, ethanolic leaf extract and aqueous leaf extract was 25µg/ml, 25µg/ml, 25µg/ml & 50µg/ml respectively.

MIC for porphyromonas gingivalis with ethanolic fruit extract was 25µg/ml with aqueous fruit extract, ethanolic leaf extract 50µg/ml and with aqueous leaf extract it showed resistance throughout.

MIC for Prevotella intermedia with ethanolic fruit extract and ethanolic leaf extract was 75µg/ml, with aqueous leaf extract MIC was 50µg/ml and with aqueous fruit extract it showed resistance throughout.

MIC for fusobacterium nucleatum with ethanolic fruit extract and ethanolic leaf extract was 10µg/ml, with aqueous fruit extract MIC was 75µg/ml and with aqueous leaf extract it showed resistance throughout. This concludes that the phytochemicals extracted from unripe papaya fruit and leaves has antimicrobial effect on A.actinomycetemcomitans, P.gingivalis, P.intermedia, F.nucleatum.

**KEYWORDS:** *carica papaya*, periodontal pathogens, well dilution method.

## INTRODUCTION

Herbal medicine has provided many of the most useful and vast variety of drugs to the modern medical science.[1] Plants have the major advantage of still being the most effective and cheaper alternative sources of drugs.[2]

The search for newer sources of antibiotics is a global challenge preoccupying research field, since many bacteria are developing resistance to synthetic drugs.[3]

Carica papaya linn is the most widely cultivated and well known member of carica linn genera belonging to family caricaceae.[4]

The latex, ripe fruits, unripe fruits, seeds, leaves, stem, bark are used as antibacterial, anti fungal, anti malarial, anti helminthic, hepatoprotective, male and female anti fertility agents.[4]

Periodontal disease is a multifactorial disease, microorganisms are considered as one of the most important factors which play a crucial role in the initiation and progression of periodontal disease. Eradication of these pathogens is necessary to control the destructive process.

Hence the aim of the current study is to.

To evaluate the antimicrobial efficacy of ethanolic and aqueous extracts made from unripe papaya fruit against periodontopathogens.

To evaluate the antimicrobial efficacy of ethanolic and aqueous extracts made from papaya leaves against periodontopathogens.

**Preparation of Extracts**

Uniform homogenized paste of unripe papaya fruit and shade dried and powdered papaya leaves were used for extract preparation.

**Cold maceration method**

**Aqueous papaya extract**

Homogenized papaya paste of 50 g was mixed with 250 ml of double standard distilled water in a glass container to obtain a homogenous mix by stirring it occasionally for 4 days at 3-5°C. The mixture was then filtered and further centrifuged at 10,000 rpm for 20 min. The supernatant was filtered through a 0.2-mm pore size Wattman filter paper grade 1 to remove any impurities (fig -1). Aliquots were stored at -20°C until required. The filtrate thus obtained was the aqueous garlic extract (AGE). This liquid extract was used for antimicrobial assay.

Similar method was followed for aqueous leaf extract wherein 50gms of powdered papaya leaf was added to 250ml distilled water and the above mentioned procedure is repeated (fig -2).

**Ethanolic papaya extract**

Homogenized papaya paste of 50 g was mixed with 250 ml of ethyl alcohol in a glass container to obtain a homogenous mix by stirring it occasionally for 4 days at 3-5°C. The mixture was then filtered and further centrifuged at 10,000 rpm for 20 min. The supernatant was filtered through a 0.2-mm pore size Wattman filter paper grade 1 to remove any impurities (fig-3).

Similar method was followed for ethanolic leaf extract wherein 50 gms of powdered papaya leaf was added to 250 ml distilled water and the above mentioned procedure is repeated (fig-4).

Stock cultures were obtained and microbiological tests were carried out at Department of Microbiology, Maratha Mandal's NGH Institute of Dental Sciences & Research Centre, Belgaum.

## METHOD

### Agar diffusion procedure

### Inoculum preparations

The colonies were transferred from the plates to the BHI broth with a sterilized straight nichrome wire. The turbidity was visually adjusted with BHI broth to equal that of a 0.5 MacFarland unit turbidity standard that has been freshly prepared. Alternatively, the suspension was standardized with a photometric device.

### Inoculation of agar plate

After adjusting the inoculum to a 0.5 MacFarland unit turbidity standard, a sterile cotton swab was dipped into the inoculum and rotated against the wall of the tube above the liquid to remove excess inoculum. Entire surface of kanamycin blood agar plate was swabbed three times, rotating plates approximately 60° between streaking to ensure even distribution. The inoculated plate was allowed to stand for at least 3 min but no longer than 15 min before punching the wells in the agar plate.

A hollow tube of 5 mm diameter was taken and heated. It was pressed on the inoculated agar plate and removed immediately after making a well in the plate. Likewise, three wells were made on each plate.

75 µl, 50 µl, and 25 µl of the 500 µl/ml of AGE and 500 µl/ml of EGE were added into the respective wells on each plate. The plates were incubated within 15 min of compound application for 18-24 h at 37°C anaerobically. The plates were read only if the lawn of growth was confluent or nearly confluent. The diameter of the inhibition zone was measured to nearest whole millimeter by holding the calipers.

## RESULTS

The results varied amongst extracts and strains.

Aggregatibacter actinomycetemcomitans showed sensitivity to ethanolic leaf, aq. leaf and aq. fruit extracts at 25µg/ml ( 12mm, 10mm, 8mm respectively) and in contrast was resistant to ethanolic fruit extract for which sensitivity appeared at a concentration of 50 µg/ml (10mm) and the size of the zone of inhibition increased as the concentration increased (fig-5).

Porphyromonas gingivalis exhibited sensitivity to ethanolic fruit extract at 25 µg/ml (8mm) but it was resistant to all other extracts at the same concentration, it showed sensitivity to

ethanolic leaf and aq. Fruit extracts at 50 µg/ml (10mm, 8mm, respectively ) but it was resistant to aq. leaf extract throughout all concentrations tested. (fig-6)

Prevotella intermedia showed sensitivity to aq. leaf extract at concentration of 50µg/ml (8mm). Whereas it was resistant to all other extracts at this concentrations. It showed sensitivity to ethanolic leaf and ethanolic fruit extracts at 75µg/ml (8mm, 8mm respectively), whereas it was resistant to aq. fruit extract throughout all concentrations tested (fig-7).

Fusobacterium nucleatum has started showing sensitivity to ethanolic leaf and ethanolic fruit extracts at a concentration of 10µg/ml (8mm, 8mm respectively) , it was resistant to other extracts at this concentration. It has started showing sensitivity to aq.fruit extract at a concentration of 75 µg/ml (8mm). It was resistant to aq.leaf extract throughout all concentrations tested (fig-8)

Results are presented in tables with respect to each extract tested (tables 1-4).

**FIGURES**

**Fig 1: Aqueous fruit extract**

**Fig 2: Aqueous leaf extract**

**Fig 3: Ethanolic fruit extract**

**Fig 4: Ethanolic leaf extract**

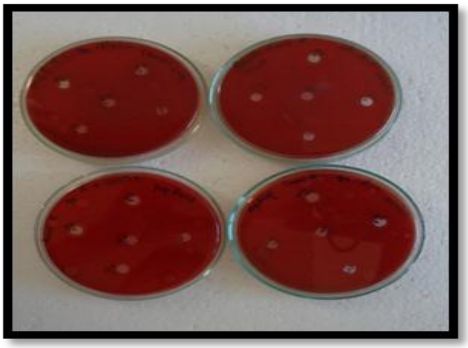

**Fig 5: agar plates inoculated with A.a showing sensitivity for extracts tested**

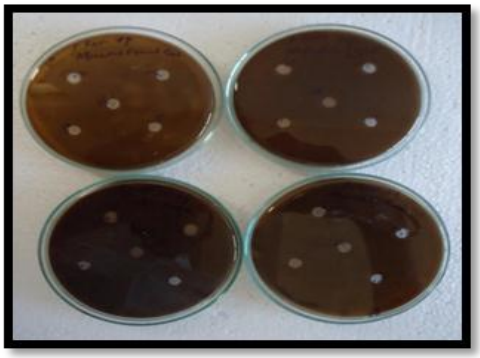

**Fig 6: agar plates inoculated with P.g showing sensitivity for extracts tested**

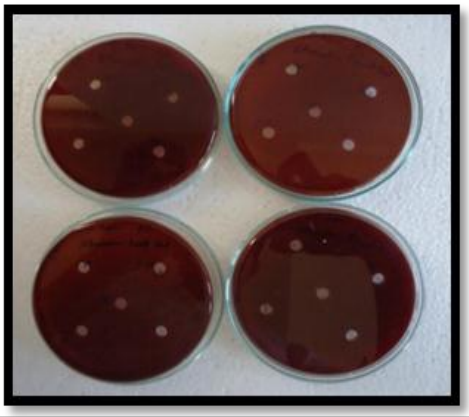

**Fig 7: agar plates inoculated with P.i showing sensitivity for extracts tested**

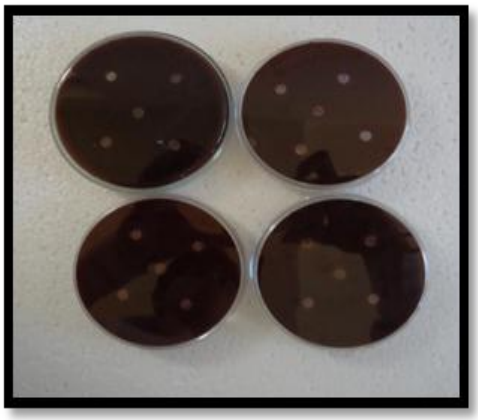

**Fig 8: agar plates inoculated with F.n showing sensitivity for extracts tested**

## TABLES

**Table 1: Sensitivity of periodontal pathogens to Ethanolic leaf extract.**

| Bacterial strains | 75µg/ml | 50µg/ml | 25µg/ml | 10µg/ml | 5µg/ml |
|---|---|---|---|---|---|
| A.a | 18mm | 14 | 12 | 0 | 0 |
| P.g | 13 | 10 | 0 | 0 | 0 |
| P.I | 8 | 0 | 0 | 0 | 0 |
| F.n | 15 | 13 | 11 | 8 | 0 |

**Table 2: Sensitivity of periodontal pathogens to Aqueous leaf extract.**

| | 75µg/ml | 50µg/ml | 25µg/ml | 10µg/ml | 5µg/ml |
|---|---|---|---|---|---|
| A.a | 17mm | 14 | 10 | 0 | 0 |
| P.g | 0 | 0 | 0 | 0 | 0 |
| P.I | 13 | 8 | 0 | 0 | 0 |
| F.n | 0 | 0 | 0 | 0 | 0 |

**Table 3: Sensitivity of periodontal pathogens to Ethanolic fruit extract.**

| | 75µg/ml | 50µg/ml | 25µg/ml | 10µg/ml | 5µg/ml |
|---|---|---|---|---|---|
| A.a | 15mm | 10 | 0 | 0 | 0 |
| P.g | 15 | 12 | 8 | 0 | 0 |
| P.I | 8 | 0 | 0 | 0 | 0 |
| F.n | 14 | 13 | 11 | 8 | 0 |

**Table 4: Sensitivity of periodontal pathogens to Aqueous fruit extract.**

| | 75µg/ml | 50µg/ml | 25µg/ml | 10µg/ml | 5µg/ml |
|---|---|---|---|---|---|
| A.a | 13mm | 11 | 8 | 0 | 0 |
| P.g | 11 | 8 | 0 | 0 | 0 |
| P.I | 0 | 0 | 0 | 0 | 0 |
| F.n | 8 | 0 | 0 | 0 | 0 |

## DISCUSSION

The results show that phytochemicals extracted from unripe fruit and leaves of carica papaya have antimicrobial effect on the aforesaid periodontopathogens.

Medline search has shown no previously conducted similar studies, hence appears to be the first study done to find out the antimicrobial effect of carica papaya on periodontopathogens.

However, an in-vitro study done by using fermented papaya preparation "FPP" (a natural health product) showed its inhibitory effect against oral microbiota like streptococcus mutans, streptococcus mitis, lactobacillus acidophilus.[5]

An in- vitro study conducted using papaya fruit extracts has shown antibacterial property against gram positive and gram negative bacteria and the bactericidal substance present in the extract, showed the properties of a protien.[6]

The phytochemical analysis of papaya showed the presence of protien, carbohydrate, flavonoids (hydroxylated phenolic substances known to have antibacterial properties), glycosides, steroids and reducing sugars.[7]

Papain, seen in the latex of adult green papaya leaves and fruits , is an endoprotein similar to human pepsin and has bactericidal, bacteriostatic and antiinflammatory characteristics.[8]
The anti microbial property of carica papaya could be attributed to papain.

## CONCLUSION

The study suggests that the phytochemicals extracted from unripe papaya fruit and leaves has antimicrobial effect on A.actinomycetemcomitans, P.gingivalis, P.intermedia, F.nucleatum.

Thus, it might be a fruitful alternative against periodonto -pathogens, however further studies need to be conducted in-vivo to conclude its clinical use as an effective antimicrobial.

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