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ASSESSMENT OF ANTIBACTERIAL, ANTI-INFLAMMATORY AND ANTIOXIDANT EFFECTS OF CARICA PAPAYA - AN IN-VITRO STUDY

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ABSTRACT

Aim: To assess the antibacterial, anti-inflammatory and antioxidant activity of Carica papaya pulp extract under laboratory condition. **Method**: In the present study, the methanolic extract of Carica papaya pulp was assessed for its antibacterial effects against *S.mutans, S.sanguis* and *S.aureus* using agar disc diffusion method and minimum inhibition concentration was determined by micro broth dilution technique. The anti-inflammatory effect was assesses by lipoxygenase inhibition assay and hyaluronidase inhibition assay. The antioxidant effects were assessed by ABTS 2, 2'-azino-bis (ethylbenzothiazoline-6-sulphonic acid) radical scavenging assay and 2, 2 diphenyl–1 picrylhydrazyl (DPPH) antioxidant assay. This was done in triplicates for each concentration of the extracts. The statistical analysis was done by Student unpaired t-test. **Results**: The methanolic extract of Carica papaya pulp extract showed no inhibitory effects on *S.mutans, S.sanguis* and *S.aureus.* The test results of DPPH assay revealed that the quercetin group had higher radical scavenging property as compared to Carica papaya group and the difference was statistically significant. The results obtained from the ABTS assay revealed that the quercetin group had higher radical scavenging property with as compared to Carica papaya. The difference in results obtained were statistically significant.($p<0.05$). The test results of lipoxygenase inhibition assay revealed that the Indomethacin group showed higher percentage of lipoxygenase inhibition activity. Hyaluronidase inhibition assay revealed that the Cromolyn group showed higher percentage of hyaluronidase inhibition activity compared to Carica papaya extract and the difference was statistically significant. **Conclusion:** Carica papaya has no antibacterial effect and insignificant anti-inflammatory and antioxidant property. Hence; further studies with large sample size and under in-vivo and vitro conditions have to be conducted.

KEYWORDS: Periodontitis, Carica papaya, lipoxygenase, hyaluronidase, antioxidant, anti-inflammatory, DPPH, ABTS.

INTRODUCTION

Periodontitis is a multifactorial disease that affects hard and soft tissues of periodontium with or without microbial colonization leading to inflammatory responses and activation of adaptive immune responses which is modified by various risk factors.[1] The success of periodontal therapy depends upon dealing with negative environment and behavioural factor and the reduction or elimination of periodontal pathogens.[2] Efficacy of periodontal treatment may be assessed by its ability to control these microorganisms.[3] Mechanical therapies, including scaling root planning and surgical procedure are aimed at improving clinical conditions by lowering microbial load either by physical removal of plaque or by radical alteration of the sub gingival habitat. Along with these mechanical therapy various antimicrobial approaches, including the use of systematically and locally administered antibiotics, directly targets subgingival species residing in the plaque biofilm or in the adjacent epithelial tissues lining the periodontal pocket.[4]

The search for newer sources of antibiotics is a global challenge pre-occupying research institutions, pharmaceutical companies and academia, since many infectious agents are becoming resistant to synthetic drugs. The situation has further been complicated with the rapid development of multidrug resistance by the microorganisms to the antimicrobial agents available. This has brought about researches using extracts from roots, herbs and fruits of plants. Plants have the major advantage of still being the most effective and cheaper alternative sources of drugs.[6]

Synthetic antimicrobial agents and antibiotics are known to cause antimicrobial resistance and emergence of opportunistic infections due to the inappropriate or widespread inadvertent use of antimicrobials. Natural phytochemicals such as *Azadirachta Indica, Aloe Vera, Guava, Calendula, Curcuma Zedoaria* and other herbs have proven to be good alternatives to such synthetic agents.[7] Natural products are rich source for discovery of new drugs because of their chemical diversity.[5] Traditional medicine has remained as the most affordable and easily accessible source of treatment in the primary healthcare system.[7]

Carica Papaya (paw-paw) is a monosexual plant of Central American origin and is also cultivated in the tropics. It is edible and good has medicinal properties. Papain, the enzyme

seen in papaya has been said to aid in digestion, assist in treatment of cancer, reduce inflammation, prevent complications of diabetes mellitus, treatment of psoriasis, chronic skin ulcers, ringworms and in prevention of Human Papilloma Virus (HPV) and as well possess antibacterial activities.[8]

The present study explores the effective concentration of Carica Papaya pulp extract required to inhibit three oral microorganisms Streptococcus mutans, Streptococcus sanguis, and Staphylococcus aureus. Also, anti-inflammatory and antioxidant efficacy of Carica papaya was assessed under laboratory conditions.

MATERIAL AND METHODS

This was an in-vitro study conducted to assess the antibacterial, anti-inflammatory and antioxidant activity of methanolic extract of *Carica Papaya*. The Ethical clearance for the study was obtained from the ethical committee and review board of the institution. Carica Papaya was procured from the local market and laboratory procedures were conducted in Skanda Life Sciences Private Limited, Bangalore.

Carica Papaya fruit pulp was cut into small pieces, dried in shade. This dehydrated fruit pulp was ground with the help of mortar and pestle (fig 1). This ground fruit pulp was used to prepare the fruit extract using methanol. The pure extract obtained was further used for assessing antimicrobial activity by disc diffusion method and MIC determination against the bacteria by micro broth dilution technique, anti-inflammatory property by lipoxygenase inhibition assay and hyaluronidase inhibition assay, and antioxidant property using ABTS radical scavenging assay and DPPH assay.

Test organisms/cultures used were *Streptococcus mutans (*ATCC 25175), *Staphylococcus aureus (*MTCC 7443) and *Streptococcus sanguis (*ATCC 10556). Chlorhexedine was used as a positive control while methanol was used a negative control. Cell suspension was prepared from *Streptococcus sanguis, Streptococcus mutans* and *Staphylococcus aureus* cultures grown on Trypticase soya broth adjusted to 1-2 x 10^5 cells/ml. 100µl inoculum of test cultures was then inoculated on Muller Hinton Agar plates for bacterial cultures. The standard disks containing the test compound (Carica Papaya fruit pulp extract), positive and negative control were placed on Agar plates. The plates were then incubated at 35^0C for 24-48 hrs. After the incubation was done the agar plates were observed for zone of inhibition around the disk.[12]

MIC determination against the bacteria by micro broth dilution technique involves Cell suspension prepared from bacterial cultures grown on Trypticase soya broth were adjusted to 1-2x10^5cells/ml. 90 µl test compounds were then mixed with different test concentration (16, 32, 64, 128, 256, 512 and 1024mg/ml) with 10 µl inoculum in 96 well plates in triplicates. Chlorhexidine was used as the positive control. The bacterial cultures were then incubated at 35^0C. After the incubation process, the bacterial test plates were observed after 24-48 hrs. Optical density (OD) was measured in Tecan plate reader at 600 nm. MIC is determined as Minimum concentration of drug giving 50% inhibition of OD as compared with control.[12]

For Anti-inflammatory activity using Lipoxygenase inhibition assay, in the test tube 0.8ml of test solution / reference standard (Indomethacin, 300 µg/ml dissolved in 3% methanol) of various concentrations, 0.1 ml of 2 M Borate buffer; pH 9.0 and 0.1 ml of 500-1000 units' Lipoxygenase enzyme. The tubes were mixed and incubated at room temperature for 5 min, after which, 2.0 ml of substrate solution (50 mg of linoleic acid dissolved in 50 µl Tween 20, then made up to 50 ml with 2M Borate buffer and diluted to a concentration of 166.6 µg/ml with 2M Borate buffer pH 9.0) were added, and mixed well . The absorbance was then measured for 4 min at 234 nm. Basal and vehicle (phosphate buffer solution (PBS) and solvent) control reaction was carried out without test sample.[13]

For Anti-inflammatory activity using Hyaluronidase inhibition assay, 0.1 M acetate buffer was prepared by mixing 44ml of Acetic acid (0.1 M) with 6 ml of Sodium acetate (0.1 M) and made up to 100 ml with de-ionized water. Hyaluronidase was activated by incubating 100µl hyaluronidase (4.15 mg/ml in 0.1 M acetate buffer, pH 3.8) with 50µl sodium chloride (26.3 mg/ml in 0.1 M acetate buffer pH 3.8) for 20 minutes at 37°C. Following activation, the enzyme mixture was pre-incubated with 200µl of test samples/reference standard at various concentrations for 20 minutes at 37ºC. Following pre-incubation, 150µl of sodium hyaluronate (6 mg/ml in 0.1 M acetate buffer pH 3.8) was added and the reaction mixture was incubated at 370C for 40 minutes. The reaction was stopped by addition 0.1 ml (0.4 N) sodium hydroxide and 100 µl (0.8 M) potassium tetraborate. This was followed by heating the mixture at 100°C for 3 minutes. The mixture was cooled and 3 ml of 67mM DMAB (P-dimethyl amino benzaldehyde) was then added and incubated at 37°C for 20 minutes. The absorbance was measured at 585 nm.

For Anti-oxidant activity using ABTS radical scavenging assay 38.4mg of ABTS was dissolved in PBS(phosphate buffer saline) and the volume was made upto 10ml. 5.59 mg of

APS (Ammonium per sulfate) was dissolved in PBS and the volume was made up to 10ml. 10ml of ABTS (7mM) and 10ml of APS (2.45mM) solutions were mixed and allowed to maintain at room temperature in dark for 16 hours. ABTS radical cations were produced by reacting ABTS and APS on incubating the mixture at room temperature in dark for 16 hours. The solution thus obtained was further diluted with PBS to give an absorbance of 1. Different concentrations of the test sample and the reference standard (quercetin) were added to 950 µl of ABTS working solution to give a final volume of 1ml, made up by adding PBS. The absorbance was recorded immediately at 734nm. The percent inhibition was calculated at different concentrations and the IC50 values were calculated by Log-Probit analysis.[13]

For Anti-oxidant activity using DPPH assay, 90µl of DPPH solution was treated with 180 µl of various concentration of test solution. The different concentrations tested for reference standard were 0.5, 1.0, 1.5, 2.0, 2.5 mcg/ml. The reaction mixture was mixed and incubated at 25°C for 15 minutes and the absorbance was measured at 510 nm using semi-autoanalyzer. Quercetin was used as a positive control. A negative control reaction was carried out without the test sample.[13]

RESULTS

The results of screening test for the antimicrobial activity carried out by disc diffusion method revealed that the chlorhexidine exhibited a zone of inhibition with the diameter of 27.3±2.1, 21.7±1.5 and 27.0±1 for *Streptococcus mutans*, *Streptococcus sanguis* and *Staphylococcus aureus* respectively, whereas. the Carica papaya pulp extract didn't exhibit any zone of inhibition. The test results revealed that the chlorhexidine showed mean % inhibition for *Streptococcus mutans*, *Streptococcus sanguis* and *Staphylococcus aureus to be* 65.02±31.26, 65.88±32.65 and 60.93±32.47 respectively. Whereas Carica papaya showed percentage inhibition of only 22.87±23.92 for *streptococcus sanguis*, 19.31±19.98 for staphylococcus *aureus* and 22.21±23.52 for *streptococcus mutans*. The mean difference in result includes 38.06 for *streptococcus sanguis*, 46.57 for *staphylococcus aureus* and 42.81 for *streptococcus mutans*. And all the difference between chlorhexedine and papaya is statistically significant ($p<0.05$). (Graph I).

In determination of anti-inflammatory effect by lipoxygenase inhibition assay, test revealed that the Indomethacin group showed higher anti-inflammatory activity with mean of 35.66±21.61 compared to Carica papaya pulp extract that has mean anti inflammatory action

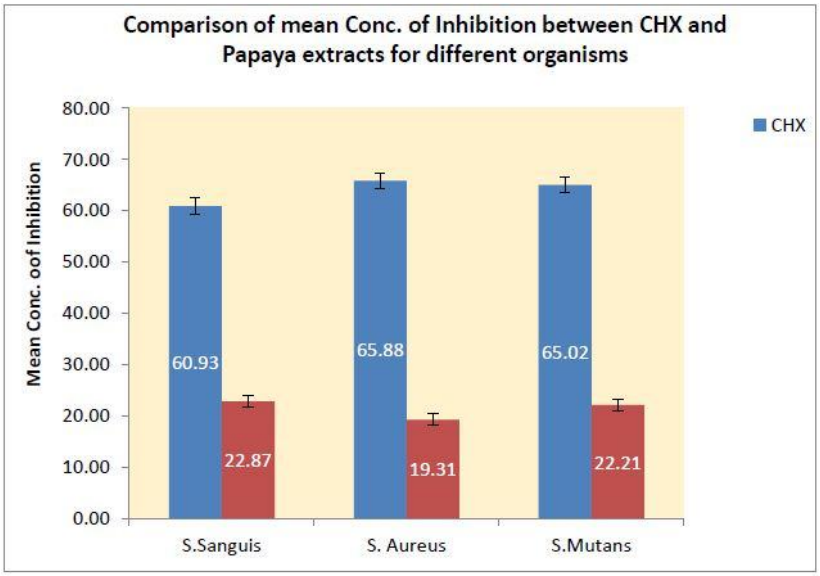
of 8.60±7.5 with a mean difference of 27.07. The difference was statistically significant (P value <0.05). (Graph -II).

In hyaluronidase inhibition assay, the test results revealed that the Cromolyn group showed good anti inflammatory action but papaya extract showed no activity. Hence; comparison was not performed for hyaluronidase assay.

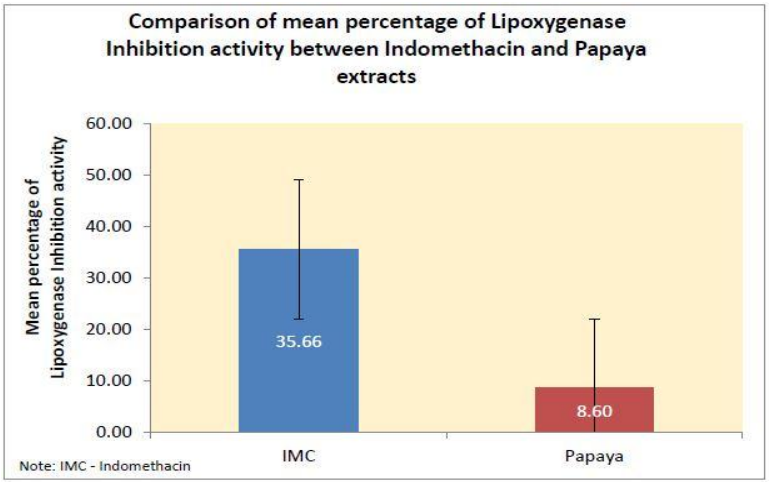
A student t test was used to in order to compare the absorbance between Quercetin group and Carica papaya group at various trials in the DPPH and ABTS assay. In DPPH antioxidant assay, the test results revealed that the Quercetin group showed higher percentage of radical scavenging action compared to Carica papaya extract. The mean value for Quercetin was 34.52±29.34 whereas 17.34±8.04 for Carica papaya. The mean difference between them was 17.17. However, the difference was not statistically significant (P>0.05). (Graph IV).

In ABTS antioxidant assay; test results revealed that Carica papaya group had lower mean percentage of radical scavenging action of 11.69±11.06 as compared to quercetin with a mean of 41.14±35.26 with a mean difference of 29.45. However, this mean difference could not yield statistically significant result (p >0.05). Hence, the absorbance did not significantly differ between two groups. (Graph III)

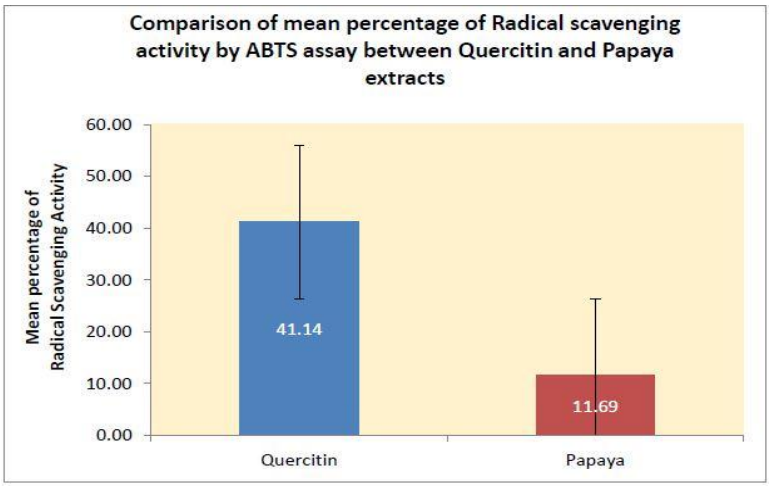
Tables and graphs



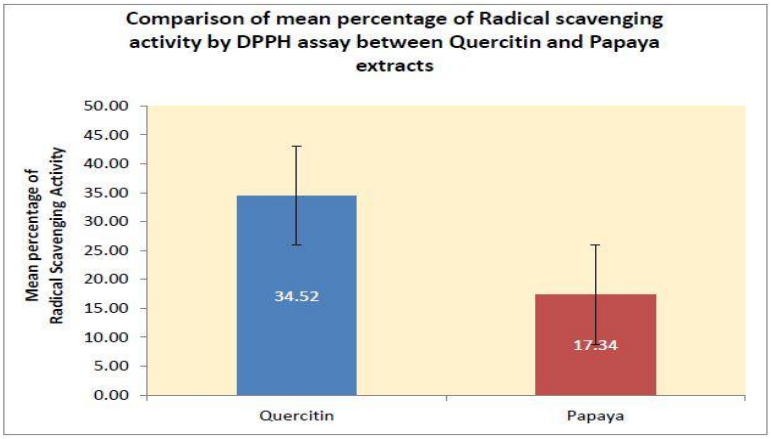
Graph I: Comparison of mean percentage of inhibition between chlorhexedine and papaya extract for S. sanguis, S. aureus and S. mutans.



Group II: Comparison of mean percentage of Lipoxygenase inhibition activity by Indomethacin (IMC) and *Carica Papaya* groups.



Graph III: Comparison of the absorbance between Quercetin (QCT) carica papaya groups at different trials by using ABTS assay.



Group IV: Comparison of the radical scavenging action between Quercetin and Carica Papaya groups at different time intervals by using DPPH assay.

DISCUSSION

The etiopathogenesis of periodontal disease is a complex process and involves both, the presence of microbial plaque and a susceptible host for the initiation and the progression of the disease. Microbial plaque formation occurs in two stages, primary colonization and secondary colonization. Gram positive bacteria such as *Streptococci sanguis, Streptococcus mitis, Streptococcus oralis* and *Staphylococcus species* forms around 90% of the bacteria. The primary colonizers attach to the teeth surfaces by cell wall structures such as fimbria, cilia etc. These primary colonizers provide the suitable environment for the attachment of more virulent gram negative periodontopathic bacteria. Also these primary colonizers prevent the penetration of host antibodies and other antimicrobial agents by the production of extracellular matrix which aid in unimpeded accumulation of microbial plaque with more virulent bacteria. Followed by the primary colonization of the bacteria, secondary colonizers such as *Porphyromonas gingivalis, Fusobacterium species, Camphylobactor species* etc. attaches to the already formed plaque matrix and matures to form a more organized dental biofilm.[14]

The periodontal pathogens produces lipopolysaccharides, endotoxins, and other virulence factors which stimulates the production of catabolic cytokines and inflammatory mediators including arachidonic acid metabolites such as prostaglandin E (PGE), Interleukin-1 (IL1), Interleukin-6 (IL6), TNF-α (Tumor Necrosis Factor-α). These cytokines and inflammatory mediators stimulate the release of tissue derived enzymes, the matrix metalloproteinase's, which cause destruction of the extracellular matrix and bone. Also with the increased inflammatory challenge there is increase in reactive oxygen species (ROS) formation in the tissues that results in the increased severity of destruction of periodontal tissues.[14,15]

The main aim of a periodontal therapy is to reduce the microbial load followed by the alteration in the host immune response to reduce the severity of destruction of periodontal tissues by reducing or altering the host derived inflammatory mediators and also free radicals produced in the tissues. Plaque control measures primarily act by reducing the microbial load and forms the indispensable part of periodontal therapy. Plaque control measures include mechanical and chemical methods and mechanical plaque control measures have been considered as a gold standard. However, mechanical plaque control measures have few limitations such as it is dependent on patient compliance, physical dexterity, inadequate accessibility, presence of anatomic aberrations etc. To overcome the drawbacks of

mechanical plaque control measures, chemical plaque control measures, local and systemic delivery of various antibiotics, lasers therapy etc. have been introduced.

The long term application of systemic antibiotics and other chemical agents results in local and systemic side effects, such as staining of teeth, taste perturbations, changes in the normal flora of the oral cavity, development of drug resistance, occurrence of opportunistic infections, gastrointestinal disturbances, drug interactions etc. Thus, in the recent years there is increased necessity for the alternative medicine with less side effects and which is cost effective also. Good oral health and periodontal health maintenance has become a great concern among all individuals and there is an increasing scientific research which is carried out in the recent years for the management and prevention of periodontal diseases. Hence, more research has been undertaken in the field of medicine for the exploration of natural products with the equal efficacy as that of chemical agents. Investigations in the field of phytochemicals have been restarted in the recent years. The use of herbal medicine dates back to ancient times and many individuals residing in the developing countries believe in the efficacy of herbal medications for the primary care.[16,17]

The papaya tree belongs to family caricaceae having four genera in the world. Carica papaya Linn is the most widely cultivated fruit in India. The fruit leaves and latex obtained from this plant is widely used for medicinal purposes.[18] It also contain a broad range of phytochemicals like polysaccharide, vitamin, mineral, enzymes, protein, alkaloid, glycosides, fats and oil, lecithin, saponins, flavanoids, sterols etc. It has significant amount of carotene that is mostly present in ripe papaya. It also contain high amount of vitamin A and C that improves eyesight.[18]

The ripened pulp of papaya has been used to treat a range of chronic diseases like dysentery, chronic diarrhea, psoriasis, bleeding piles, urinary tract wound, obesity etc.[18] The seeds and pulp of papaya has shown antibacterial action against *Bacillus subtilis, Enterobacter cloacae, Escherechia coli, Salmonella typhi, Staphylococcus aureus, Proteus vulgaris*. The effective antioxidant supplementation bionormalizer, made from fermented papaya available in Japan have shown to improve the haemorrheology in alcoholic individuals.[19]

Various studies on Carica papaya have shown strong antimicrobial properties against both Gram-positive and Gram-negative bacteria. However, in the literature a few studies have been evaluated the antimicrobial property of Carica papaya on oral bacterial species. Thus, the

present study was carried out to assess the antimicrobial property of Carica papaya pulp methanolic extract against the most common oral bacteria i.e. *Streptococcus mutans* (ATCC 25175), *Streptococcus sanguis* (ATCC 10556) and *Staphylococcus aureus* (MTCC 7443) anti-inflammatory and antioxidant property under the laboratory conditions.

For the assessment of antimicrobial property these three organisms were selected, as these are the first ones to colonize the oral cavity and provide the suitable environment for the development and growth of more invasive Gram-negative bacteria in the microbial plaque or biofilm. Also, these organisms have been found to be the main etiological agents in the initiation of dental caries and periodontal diseases.[22] Most of the antimicrobial compounds obtained from Carica papaya are often obtained through the initial ethanol and or methanol extraction method. Hence, in the present study methanolic extraction method was selected for obtaining the active ingredient of Carica papaya pulp.[20]

According to the results obtained from this study, the methanolic extract of Carica papaya pulp extract had no inhibitory effects on growth of *Streptococcus mutans, Streptococcus sanguis* and *Staphylococcus aureus.* The positive control chlorhexidine showed the maximum zone of inhibition of 27.3±2.1, 21.7±1.5 and 27.0±1 for *Streptococcus mutans, Streptococcus sanguis* and *Staphylococcus aureus* respectively. The negative control methanol did not show any inhibitory effects on all the three organisms. After screening for the zone of inhibition the minimum inhibitory concentration of Carica papaya pulp extract was assessed and compared with that of positive control chlorhexidine. Chlorhexedine showed mean % inhibition for *Streptococcus mutans, Streptococcus sanguis* and *Staphylococcus aureus to be* 65.02±31.26, 65.88±32.65 and 60.93±32.47 respectively. Whereas Carica papaya showed percentage inhibition of only 22.87±23.92 for *streptococcus sanguis*, 19.31±19.98 for staphylococcus *aureus* and 22.21±23.52 for *streptococcus mutans*. The mean difference in result includes 38.06 for *Streptococcus sanguis*, 46.57 for *Staphylococcus aureus* and 42.81 for *Streptococcus mutans*. All the difference between Chlorhexedine and papaya was found to be statistically significant ($P<0.05$). Hence; the effect of papaya on the micro organism was almost negligible. The results obtained from this study were similar to the results of the study conducted by Vieira et al.[10]

The results were in contrast to Akujobi et al[7] and Somanh et al[8] who found that papaya extract was effective in inhibiting the growth of *Streptococcus aureus* and *Streptococcus mutans*. This may be because of difference in the composition of the different parts of the

plant. The composition of extract is also influenced by the geographical location of the plant, age of the plant, season of harvesting, growth stage, and methods of drying and extraction technique. Also, the different parts of the plant have varying level of antimicrobial activity.

The results of the lipoxygenase inhibition assay of the present study revealed that the Indomethacin group showed higher anti-inflammatory activity with mean of 35.66±21.61 compared to Carica papaya pulp extract that has mean anti inflammatory action of 8.60±7.5 with a mean difference of 27.07. The difference was statistically significant (P <0.05).

In hyaluronidase inhibition assay, the test results revealed that the Cromolyn group showed good anti inflammatory action at various concentrations whereas *Carica papaya* extract showed no anti-inflammatory action. The result obtained was in contrast to the study by Sagnia et al[17] who found that leaf extract of *Carica papaya* had shown good anti inflammatory effect. Other in vitro studies are lacking, which have studied the effects of other parts of papaya such as the fruit pulp and peel. In vivo studies by Chen et al, lin et al, Nayak et al have shown papaya to have good healing and anti inflammatory action.[21]

The results obtained in this study for the antioxidant property of *Carica papaya* pulp extract reveal that in DPPH assay the positive control Quercetin group showed higher anti oxidant action compared to Carica papaya extract. The mean value for Quercetin was 34.52±29.34 whereas 17.34±8.04 for Carica papaya. The mean difference between them was 17.17. However, the difference was not statistically significant.

The test results of ABTS assay revealed that *Carica papaya* extract had lower mean percentage of radical scavenging action of 11.69±11.06 as compared to quercetin with a mean of 41.14±35.26 with a mean difference of 29.45. However, this mean difference could not yield statistically significant result (P value=0.06). Hence, the antioxidant action did not significantly differ between two groups. The results obtained in this study are similar to the results obtained by the study conducted by Osato et al and Maisarah et al.[9,11]

CONCLUSION

The following conclusions can be drawn from the present study:

1) Methanolic extract of Carica papaya pulp had no antibacterial effects on common oral bacteria namely, Streptococcus mutans, Streptococcus sanguis and Staphylococcus aureus.

The antimicrobial efficacy was almost negligible when compared to chlorhexidine which is the positive control and considered as gold standard for its antimicrobial property.

2) The lipoxygenase assay showed low anti inflammatory action of papaya extract compared to indomethacin.

3) The hyaluronidase assay showed no anti inflammatory action of papaya extract.

4) The ABTS assay showed low antioxidant action of papaya extract compared to positive control quercetin.

5) The DPPH assay also showed lower antioxidant action of papaya extract compared to positive control quercetin.

Hence; it can be concluded that Carica papaya pulp extract only shows limited antioxidant and anti-inflammatory effect with no antibacterial action under laboratory conditions.

Limitations of the present study

The major limitation of the study was that, only three pathological oral organisms were selected for the study. It was an in-vitro study conducted to study the antimicrobial, anti-inflammatory and Antioxidant property of Carica papaya pulp extract. The outcome of the study cannot be correlated to the in vivo scenario

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