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RESEARCH ARTICLE

GUAVA LEAF EXTRACT - A CREDIBLE ANTICARIOGENIC AGENT

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ABSTRACT

Background: Dental caries remains one of the most common multifactorial infectious diseases of mankind. Prior studies have shown that the bark and leaf extracts of guava have some pharmacological activities like anti – inflammatory, anti oxidant properties, etc. Conversely, not many studies have shown the efficacy of guava leaf extract as an anti cariogenic activity. The purpose of the study was to find out whether there is an inhibitory effect of guava crude extract on microbial flora of dental caries and to compare its efficacy with kanamycin antibiotic discs.

Method: Caries samples were placed in brain heart infusion (BHI) broth and incubated for 24 hours at 37ºc. After incubation the samples were inoculated on suitable solid media and various concentrations of crude extract 8, 16 and 32 fold of the minimal inhibitory concentration (MIC) were placed and incubated for 24hours.

Results: The antibacterial activity of the guava leaf extract against the microbial flora was tested by agar diffusion method, the largest clear zone of 13.42857 mm was obtained in 32 × MIC chewable tablets, whereas the positive control kanamycin produced a clear zone of 13 mm. The crude extract at the concentration of 32× MIC provided the highest inhibitory activity against the microorganisms followed by 16× MIC and 8× MIC concentrations.

Conclusion: Though guava leaf extract appears to be efficient against dental caries, further in vivo studies need to be performed with larger sample size taking the safety dosage into consideration.

INTRODUCTION

Dental caries have overrun humans since the dawn of civilization and still constitutes one in all the foremost common human infectious disease in various parts of the world.[1] As a matter of fact individuals from developed countries are preferring to use herbal plants as a traditional medicine in various parts of day to day practices. Many herbal products have shown to improve oral health by inhibiting the formation of biofilms, reducing the adhesion of microbial pathogens to the tooth surface because of their anti bacterial & anti microbial properties. Many attempts have been made to eliminate the microbial pathogens from the oral flora. Antibiotics such as chlorhexidine, penicillin, ampicillin, tetracycline, erythromycin, kanamycin and vancomycin are very effective in preventing dental caries in vivo and in vitro. But excessive use of these antibiotics can result in alterations of the oral and intestinal flora and cause adverse effects such as development of bacterial tolerance, vomiting, diarrhea and teeth stains. These problems have initiated in further search for natural antimicrobial agents with less untoward effects. [2]

Psidium guajava (PG) commonly known as guava has been used as toothpaste in myth practices to maintain good oral hygiene. Most of the studies have shown that the bark and leaf extracts of guava have some pharmacological activities such as anti-inflammatory, anti-oxidant, antimutagenic besides antimicrobial activities. Nevertheless very few studies have shown the efficacy of guava leaf extract against caries activity**.** [2] Thus, the purpose of this study was to find out whether there is an inhibitory effect of guava crude extract on microbial flora of dental caries and to compare its efficacy with kanamycin antibiotic discs.

MATERIALS AND METHODS

Preparation of guava chewable tablets

Guava leaves were cleaned and dried in the oven at 60°c for 48 hours. The dried leaves were finely grinded into powder form. Following this, menthol and peppermint oil were added to this powder in different concentrations of 8, 16 and 32 fold of minimal inhibitory concentration (MIC).

Method of sampling

Caries samples were collected from patients and placed in brain heart infusion (BHI) broth which is the best for culturing of anaerobic microorganisms under aseptic conditions and incubated for 24 hrs at 37ºc.

Assay method

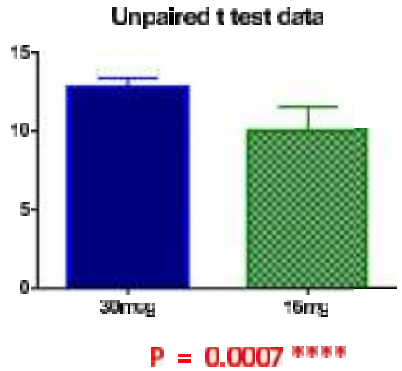
Agar diffusion method

The samples were inoculated on the suitable solid media such as Muller Hinton agar by spread method and incubated at 37ºc for overnight. The various concentrations of crude extract 8, 16 and 32 fold of the minimal inhibitory concentration (MIC) were used. After incubation, the diameter of inhibition zone for each concentration was measured and compared to the standard antibiotic (kanamycin) in order to evaluate the resistance / susceptibility based on growth of the microbial flora. [2] Following this, statistical analysis was done by using unpaired't' test and Anova single factor analysis.

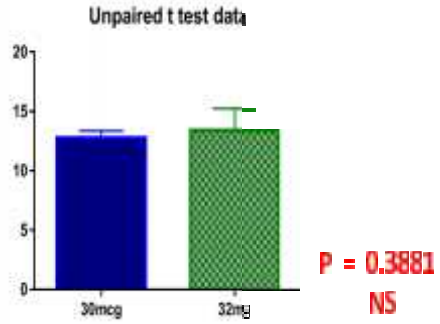
RESULTS

This study was designated to evaluate the antibacterial activity of crude extract from the leaves of P. guajava. The antibacterial activity of the extract against the microbial flora was tested by using agar diffusion method **(Fig 1).** The MIC is defined as the lowest concentration of the compound at which the microorganism tested does not demonstrate visible growth [2]. The antibacterial sensitivity was determined by agar diffusion method as described before, the largest clear zone of 13.42857 mm was obtained in 32× MIC chewable tablet, whereas the positive control, kanamycin, produced a clear zone of 13 mm **(Fig 2).**This difference was statistically not significant **(Graph 3).** In the present study, significant difference was seen between 8x MIC and 16 X MIC when compared with kanamycin **(Graph 1 and 2).** These observations indicated that the crude extract at the concentration of 32× MIC provided the highest inhibitory activity against the microorganisms followed by 16× MIC and 8× MIC concentrations **(Graph 4 and 5).**

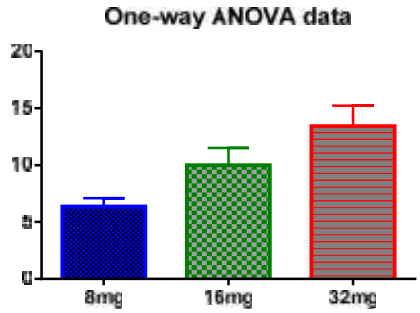
Graph 1 Comparison between 30mcg of kanamycin and 8X MIC Chewable tablet



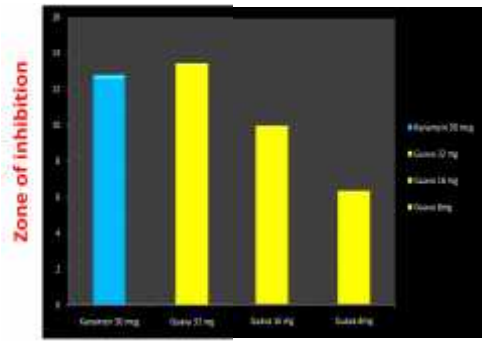
Graph 2 Comparison between 30mcg of kanamycin and 16X MIC Chewable tablet



Graph 3 Comparison between 30mcg of kanamycin and 32 X MIC Chewable tablet



Graph 4 Comparison within the groups of guava chewable tablets (8XMI, 16XMIC & 32XMIC)



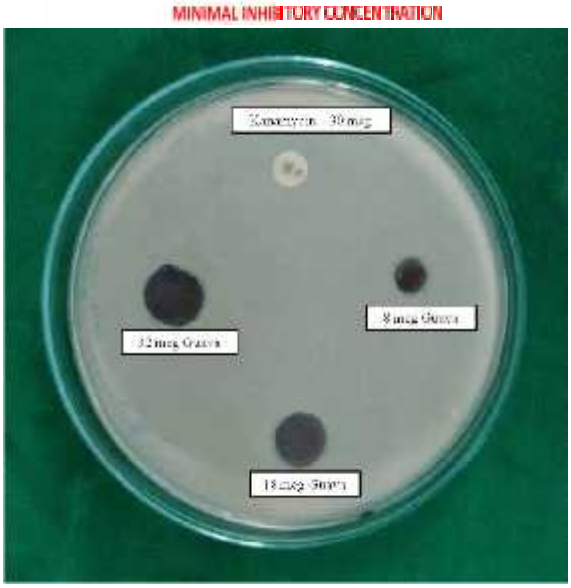


Fig 1 Kanamycin and guava chewable tablets of different concentrations in agar plate before incubation.

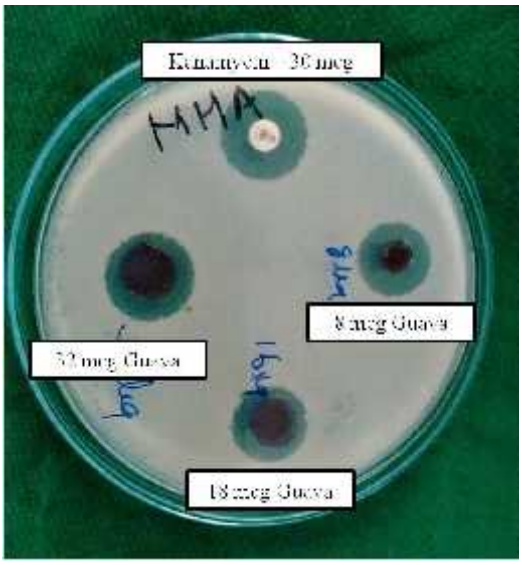


FIG 2 Zone of inhibition after 24 hours incubation

DISCUSSION

The natural plant products such as Neem, babool and miswak, etc have been proven to be the most effective biologically active compounds that are used in traditional practices in order to maintain oral hygiene. Concerning the diseases caused by the micro organisms the increasing resistance in many pathogens to commonly used antibiotics has led to the development of anti- microbial compounds derived from herbs.[3] The use of the herbal plants as a traditional medicine to maintain oral health is common in developed countries. Leaves of Psidium guajava are used in USA as an antibiotic in the form of poultice or decoction for wounds, ulcers and toothache. In Nigeria, South Africa and Kenya the leaves are used as in the treatment of the conditions like malaria, gastroenteritis, vomiting, diarrhoea, ulcers, toothache, coughs, sorethroat , inflamed gums and number of other conditions.[4]

Saraya et. al (2008) conducted a study to develop the guava extract chewable tablets for anticariogenic activity against S. mutans by agar diffusion method and concluded that 32× MIC tablet had highest growth inhibitory efficacy **(12.50 ± 0.71 mm)** against S. mutans.[2]

Garode AM and SM Waghode conducted a study to develop the antibacterial activity of guava leaves extracts against S. mutans and showed that ethanol extract of guava leaves had maximum activity with the zone of inhibition 18mm against S. mutans while remaining extracts of guava leaves like acetone, methanol and petroleum ether had moderate activity against S. mutans with zone of inhibition 12 mm, 11 mm and 12 mm respectively. The antibiotic sensitivity test, antibiotic erythromycin shown optimum activity against S. mutans (28mm). In the present study, crude extract was also tested for antimicrobial susceptibility compared with standard antibiotic kanamycin. The largest clear zone of 13.42857 mm in diameter was observed with crude 32× MIC comparing to 12.78571 mm with kanamycin.[5]

Thompson T et.al conducted a study by using phytoderivatives from Psidium guajava against the bacterial isolates by using well diffusion method. He concluded that acetone extract of Psidium guajava showed maximum inhibitory activity against B. megaterium, S. viridans with the zone of inhibition 29mm and 21mm respectively whereas its methanol extract showed high activity against N. catarrhalis (20mm). Ethanol extract inhibit the growth of P. aeruginosa (18mm) and the water extract revealed high activity against S. viridans and B. megaterium both with a zone of 15mm.[6]

Biswas B et.al have conducted a study to develop the antimicrobial activity of extracts of guava leaves on gram negative and gram positive bacteria and concluded that only two of the crude solvent extracts prepared from the leaves of Psidium guajava, methanol and ethanol, showed inhibitory activity against the gram-positive bacteria, B. cereus and S. aureus while neither of the Gram-negative bacterium showed any inhibition.[7]

As observed in the present study that 32 MIC tablet provided the best inhibition activity against the microorganisms while 16 MIC tablet provided the lower inhibition activity than 32× MIC tablet but higher than 8 MIC tablet. The possible reason for antimicrobial activity of guava leaves extract was the presence of more than 20 isolated compounds, including alkaloids, anthocyanins, carotenoids, essential oils, fatty acids, lectins, phenols, saponins, tannins, triterpenes and vitamin C. Leaf and bark extracts have in vitro antimicrobial activity mostly associated with flavonoids such as morin glycosides, quercetin and quercetin glycosides which was confirmed by the phytochemical analyses**.** The leaf extract of Psidium guavajava have trypanocidal activity which may be attributed mainly due to antimicrobial property of the flavanoids and iron chelating property of tannins. As the gram positive bacteria have a mesh-like peptidoglycan layer, is more accessible to permeation by the extracts. In addition to guava leaves extracts, presence of menthol & peppermint oil that were used as ingredients for the preparation of guava chewable tablets are active against both gram positive & gram negative bacteria as well as fungi.[7] Collectively guava chewable tablets showed the higher antimicrobial activity than kanamycin antibiotic disc.

CONCLUSION

In the present study (in vitro) based on these observations it was evident that the bioactive compounds such as quercetin, flavonoids and tannins in the guava leaf extract have bacteriostatic activity against the microbial flora. Though guava leaf extract appears to be efficient against dental caries, further in vivo studies need to be performed with larger sample size taking the safety dosage into consideration.

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