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Antimicrobial activity of *Punica granatum* and *Psidium guajava* on *Candida albicans* and *Enterococcus faecalis:* An *In-Vitro* study.

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ABSTRACT

Antimicrobial agents have been used to eliminate oral pathogens. The excessive use of conventional antimicrobial agents has resulted in microbial resistance, imbalance in oral flora and numerous adverse effects. These draw backs have provoked researchers to search an alternate novel antimicrobial agents that are safe and specific for oral pathogens. The use of plants as therapeutic agents has been gaining interest of late due to its reduced adverse effects. *E faecalis* is one of the major causes of failed RCT. while *C albicans* being an oral commensal, can cause oropharyngeal infections. To evaluate the antimicrobial activity and minimum inhibitory concentration of *Psidium guajava*, *Punica granatum* against *E. fecalis* and *C albicans* Methanolic extracts of *P granatum*and*P guajava* were tested against oral diseases causing oraganims like *Candida albicans*(ATCC- 2091) and *Enterococcus fecalis*(ATCC-35550). Antimicrobial activity was evaluated by agar well diffusion method and minimum inhibitory concentration was done for the same. *P guajava* and *P granatum* showed statistically significant antimicrobial activity against *E faecalis* and *C albicans*. *Psidium guajava* and *Punica granatum* may be used as potential antimicrobial agents against *Enterococcus fecalis* and *Candida albicans*.

Keywords: *Psidiumguajava*, *Punicagranatum,* antimicrobial activity*,* agar well diffusion. MIC*,*

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INTRODUCTION

The oral microbiota is maintained in a delicate balance in healthy individuals. Disruptions in this homoeostasis lead to a wide variety of oral diseases. The link between oral diseases and the microbial species that form part of the microbiota of the oral cavity is well established. *Streptococcus mutans*, *Lactobacilli, Porphyromonas gingivalis*, *Aggregati bacteriactinomycetemcomitans, Enterococcus faecalis*, *Candida albicans, etc are* some of the major pathogens, responsible for the common oral diseases like dental caries and periodontal diseases.

Enterococcus faecalis, a facultative anaerobe, has the ability to survive in the root canal system as a single organism without the support of other bacteria to invade and live within the dentinal tubules and are associated with failed root canal treatments.[1] *Candida* species, being commensal yeasts in healthy individuals, which is frequently isolated from mucosal surface are capable of causing oropharyngeal infection in individuals with immune deficiencies and those with severe underlying diseases like diabetes, periodontitis and in patients with removable oral prostheses.[1,2]Success of endodontic treatment depends on complete debridement of root canal space. The occurrence of fungi *C.albicans* and bacteria *E.faecalis* in failed endodontic treatment cases has been reported in several studies. [3] The pathogenic activity of dental plaque bacteria, systemic and local risk factors, resistance to the organisms contribute to the genesis and progression of periodontal disease [4].

The excessive use of antimicrobial agents results in microbial resistance [5, 6], imbalance in oral flora and numerous adverse effects. Chlrohexidine is the most commonly used antimicrobial to inhibit these microorganisms. The adverse effect of chlrohexidine outrages the benefits and hence necessitate further research for alternate novel antimicrobial agents that are safe and specific for oral pathogens. Natural phytochemicals derived from plants have proven to have high antimicrobial, anti-inflammatory, anti-oxidant and biocompatible properties.

Punica granatum and *Psidium guajava* are routinely used in daily life especially in south east Asia and they provide many benefits such as antioxidant properties and antimicrobial effects.

Punica granatum (pomegranate) is a shrub or small tree native from Asia. Pomegranate, largely due to its staggering antioxidant potential, has been touted as a potential weapon to fight a wide array of diseases. *Psidium guajava (guava*) is a tropical tree, used as an effective and reliable aid in cleaning teeth traditionally and also has anti-diarrheal, anti-inflammatory, anti-oxidant properties etc.

The aim of our study is to evaluate the antimicrobial activity of *Psidium guajava, Punica granatum, on Enterococcus faecalis and Candida albicans* by agar well Diffusion Method and determination of Minimum inhibitory concentration(MIC) by broth dilution method.

MATERIAL AND METHODS

Preparation of Extract and isolation of the organisms

The *Psidium guajava* leaf powder was procured from the NKCA Ayurvedic hospital, Mysore and fresh *punica granatum* juice was extracted from the fruit. Obtained leaf power and juice of the plants was soxhletted in 250ml of methanol solution at 80° C for 12hr. Extracts were than filtered using Whatman no 1 filter paper, dried and dissolved in 50% of dimethyl sulfoxide(DMSO) to make a stock solution which was stored at 4°C.

Strains of *E faecalis (ATCC- 35550)* and *C albicans (*ATCC- 2091) were obtained. The cultures and strains were maintained in nutrient agar slants in refrigerator at 4 °c.

Agar well diffusion method

Bacterial inoculum were prepared to match 0.5 McFarland standard.5 mm diameter wells was punched into the freshly prepared blood gar and Sabouraud Dextrose for *E faecalis* and *C albicans* respectively. All plates were inoculated by streaking the organisms on the plates with the test bacterium, Different

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concentrations of extracts from 5 µl, 10 µl, 25 µl, 50 µl and 75µl was dispensed into each well after the inoculation of the plates with bacteria. 0.2% chlorhexidine was used as reference control. Inoculated plates were sealed, labeled and incubated at 37°C for 24 hr. After incubation the plates were examined for the inhibition zones[figure 3 & 4] and the experiments were carried out in triplicates.

Minimum inhibitory concentrations(MIC) refers to the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism. Broth dilution method was done where in 9 dilutions of each drug was prepared with BHI. To determine the MIC of the extracts the starting concentration was 100 µg/ml. Serial dilutions were made with sterile saline to further achieve the following concentrations of 50, 25, 12.5, 6.25, 3.12, 1.6, 0.8, 0.4, 0.2 µg/ml

RESULTS

The zone of inhibition observed against test extracts are summarized in Table 1, figure 1, figure 2.

Table 1

	Test Compound	Concentration µg/ml	Zone of Inhibition (in mm) E faecalis	Zone of Inhibition (in mm) Candida albicans
Efaecalis	PsidiumGuajava	5	-	10
		10	8	14
		25	10	18
		50	14	20
		75	**16**	**21**
	Punicagranatum	5	-	10
		10	-	18
		25	12	20
		50	15	27
		75	**20**	**30**

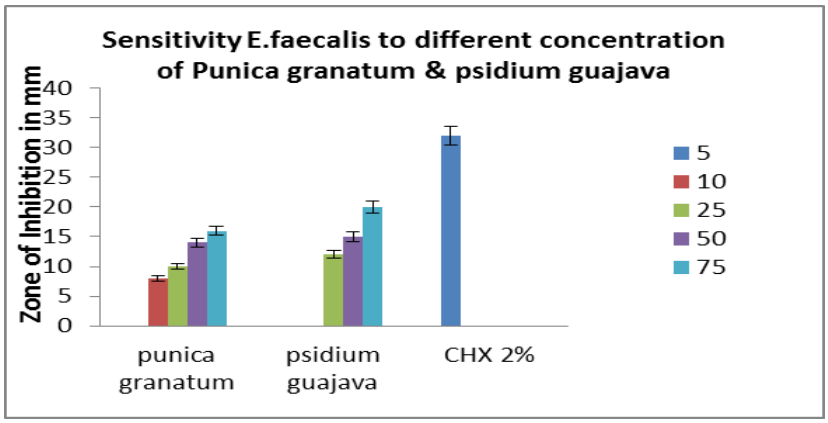

Figure 2: zone of inhibition of *punica granatum* & *Psidium guajava* on *E faecalis*

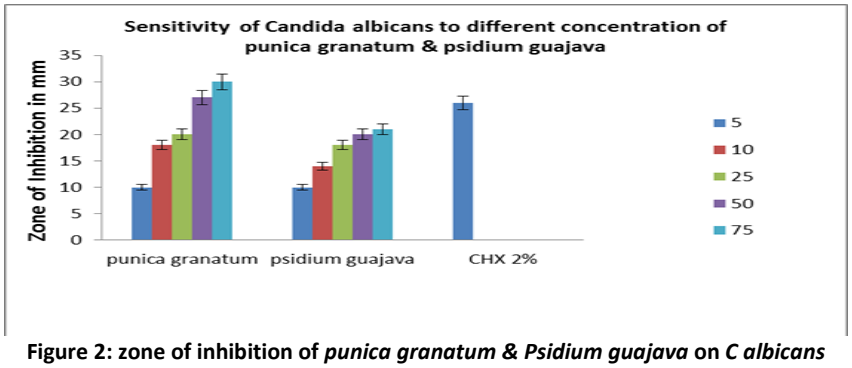

Figure 2: zone of inhibition of *punica granatum* & *Psidium guajava* on *C albicans*

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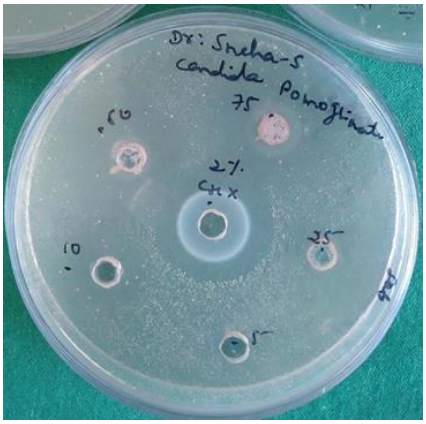

Figure 3

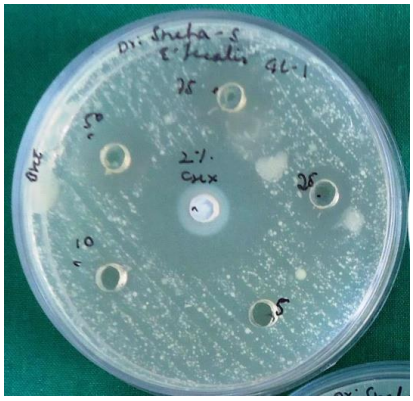

Figure 4

MIC for *P guajava* was recorded 50µg/mL for E faecalis and 1.6µg/mL for C albicans and *P granatum* showed MIC at 100µg/mL for E faecalis and 6.25µg/mL for *Calbicans*. So are results showed that both the extracts inhibited bacterial growth in that *Calbicans* inhibited at lower concentration compared to *E faecalis*.

DISCUSSION

Plant chemicals are one of the most powerful alternative chemotherapeutic agents to control many infections. *Punica granatum (L.Punicaceae)* the common name is derived from Latin word sponus and granatus.

The fruit is native to Afghanistan, Iran, China and the Indian sub- continent.[7]*Punica granatum* peel and juice contains substantial amounts of phenolic compounds, including flavonoids (anthocyanins, catechins and other complex flavonoids) and hydrolyzable tannins (punicalin, pedunculagin, punicalagin, gallic and ellagic acid), Gallic acid, ellagic acid and punicalagin, have free radical-scavenging properties, Which account for 92% of the antioxidant activity and also possess antiproliferative activity (inhibiting proliferation from 30% to 100%, against all cell lines), antifungal and antibacterial properties. [8,9]

Psidium guajava is traditionally used to manage conditions like malaria, gastroenteritis, vomiting, diarrhea, dysentery, wounds, ulcers, toothache, coughs, sore throat, inflamed gums, and other conditions. Bioactive components in the *psidium guajava* leaf can regulate blood glucose levels, can even aid in weight loss has anti-inflammatory, antidiarrheal, antioxidant, antimutagenic, besides antimicrobial activities it is suggested that the extracts may have potential for use as anti-plaque agents.[10]

Hazzani(2012) studied the in vitro antibacterial activity of homemade pomegranate aqueous extract syrup (molasses) compared to that found in the market against thirteen bacteria varying between gram

positive and gram negative. Most of the micro organisms, showed the zone of inhibition which was about greater than 15mm in diameter.[11]

Ismail(2014) made an extensive and systematic review of the literature of the fruit and peel extract of *Punica granatum.* which showed that this fruit has high antioxidant potential. *Punica granatum* has gained a wide acceptance for their pharma- cological activities against serious maladies such as prostrate, colon and liver cancers, stomach ulcers, cardiovascular diseases and digestive disorders. [12]

Amith Pandey(2012) studied the antimicrobial activity of the ethanolic , methanolic, ethyl acetate and hot water extracts from the leaves, fruit and stem of *psidium guajava* in their study to all the parts stems showed best results with the zone of inhibition being 28.5mm against few selected pathogens.[13]

In a study by S Saraya(2008) it was shown that the minimal inhibitory concentration (MIC) against *S. mutans* of crude extract was 5 mg/ml which was equal to minimum bactericidal concentration (MBC). Crude extract was also tested for antimicrobial susceptibility compared with standard antibiotic kanamycin. The largest clear zone of 12.50 ±0.71 mm in diameter was observed in crude 32× MIC comparing to 13 mm in kanamycin. [10]

In our study, the extracts of *Psidium guajava* and *Punica granatum* possessed antimicrobial activity against *E faecalis* and *C albicans*. *Psidium guajava* showed maximum zone of inhibition upto 16mm and 21mm against *E. faecalis* and *C albicans* respectively and *Punica granatum* showed maximum zone of inhibition upto 20mm and 30mm against *E. faecalis* and *C albicans* respectively.

CONCLUSION

In our study *Psidium guajava* and *Punica granatum* showed antimicrobial activity against *E faecalis* and *C albicans*. *Punica granatum* showed larger diameter of zone of inhibition against both the organisms. Ethnopharmacological utilization of these plants is prevalent in a variety of cultures to cure common disorders without any consideration to its phyto- chemical profile and adverse effect. Clinical trials are needed prior to its pharmacological exploitation by modern medicine to verify its safety and dosage for specific ailments. Large scale trials should be conducted to confirm its biological benefits which could open doors to a natural, economical way to combat oral diseases

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