AN ANTIMICROBIAL EFFICACY OF GUAVA AND TULSI AGAINST STREPTOCOCCUS MUTANS AND E. FAECALIS: IN VITRO STUDY

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ABSTRACT

Objective*: To evaluate the efficacy of alcoholic extracts of Tulsi and Guava on Streptococcus mutans and Enterococcus Faecalis.*

Methods*: An experimental design, in vitro study, in which ethanolic extract of Tulsi and Guava were prepared by Soxhlet extraction method. The extracts were then diluted with an inert solvent, normal saline, to obtain 5 different concentrations 30%, 15%, 7.5%, 3.75% and 1.88%. The extracts were then subjected to microbiological investigation to determine the concentration which would gave a wider inhibition zone against Streptococcus mutans and Enterococcus Faecalis. The zones of inhibition were measured in millimeters.*

Results*: At all concentrations Guava extract showed a wider zone of inhibition than that of Tulsi extract against Streptococcus mutans (S. mutan) and Enterococcus faecalis (E. feacalis). The*

widest zone of inhibition was observed at 30% among all five different concentrations of Tulsi and Guava that were investigated.

Conclusion: *Tulsi and Guava extract demonstrated an antimicrobial property against Streptococcus mutans and Enterococcus Faecalis.*

KEYWORDS: Antimicrobial activity, Enterococcus Faecalis, Guava, Streptococcus mutans, Tulsi, Zone of inhibition

INTRODUCTION

Oral health is an important part of our total health. A person cannot live a healthy life until his oral cavity is free of infection like dental caries, gingivitis, halitosis and periodontitis which are not uncommon to human.

Streptococcus mutan is considered as a chief and pioneer micro-organism for most of the dental infections whereas Enterococcus faecalis is the most isolated or detected species from oral infections, including marginal periodontitis, infected root canals, and peri-radicular abscesses. [1] E. faecalis is the most resistant bacteria that can easily survive in the nutrient deprive condition and had become resistant to most of the antibiotics. [2]

Modern dental interventions aimed to treat the disease but none of them target specifically to the etiology of disease. Therefore, some newer method of treating dental infection requires to be developed. An antimicrobial agent that is effective and also acceptable to young children will be a useful supplement to current techniques for the prevention of dental infection.[3] Various synthetic chemical agents have been evaluated over the years with respect to their antimicrobial effects against dental infection, however all are associated with various side effects; thus patient are going away of modern day medicines and they prefer using herbal ayurvedic preparations which are efficient with least possible side effects.[4]

In recent era, focus on phytomedicine research has increased all over the world and a large body of evidences has showed immense potential of medicinal plants used in various traditional systems. The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. The medicinal use of plants in Hindu culture was earliest

mentioned in "Rigveda", which is said to have been written between 4500 - 1600 BC and is supposed to be the oldest repository of human knowledge. The term "Danta-shastra' i.e. dentistry in ayurveda is not new; it all started with chewing sticks and has come of age to mouthwashes. [5]

Thus, the therapeutic uses of herbal preparation will provide effective, economical and safer edge against various synthetic chemical agents.

Tulsi leaves are quite effective in treating common oral infections. Carracrol and Tetpene are the antimicrobial agents present in Tulsi. [6]

In an in-vitro study the various concentrations of the Tulsi extracts have been assessed against Streptococcus mutans and concluded that the composition of Tulsi extract 4% has a maximum antimicrobial potential. [7]

Guava has the ability to inhibit the growth of the common oral flora due to the presence of secondary metabolites. And this helps it to become an alternate and also to minimize the excessive use of antibiotics for the prevention of dental caries. [8]

Though some literature is available about efficacy of the Tulsi and Guava against oral infection but none of them evaluate their efficacy against the S. mutan and E. faecalis simultaneously. Therefore, this study has been undertaken to evaluate the efficacy of various concentrations of Guava and Tulsi as an anti-microbial agent against S. mutan and E. faecalis.

MATERIALS AND METHODS

Preparation of Guava and Tulsi extract

Young leaves of Guava, Tulsi were plugged from the respective plants and leaf samples were dried in a shade and grounded in an electric blender to obtain it as a coarse powder. Ethanolic extracts were obtained through Soxhlet apparatus with ethanol as a solvent. The filtrate for each extract was concentrated using a rotavapor and freeze dried using lyophilization, after which the residues were finely grounded, weighed and stored at 4°C for further experiments.

Preparation of 5 different concentrations of Tulsi and guava extract

A stock solution (30% concentration of the extract in normal saline) was taken and 5 subsequent doubling dilutions of each extract were made to obtain concentrations of 15%, 7.5%, 3.75%, and 1.88%.

Microbiological procedures

Pure strains of Streptococcus mutans (ATCC 890) and E. faecalis were obtained from MTCC Chandigarh. Muelar hinton agar was used as a culture medium. The well diffusion method was used to determine the zone of inhibition. In this method, five circular wells that could incorporate five different concentration of the test agent (Tulsi and Guava extract) were cut in the agar plates using a template. Four plates were prepared and labeled, for the 5 different concentrations of Tulsi and Guava extract. The extract, was transferred to the respective agar plates and these incubated aerobically at 37°C for 48 h. The inhibition zones were measured using a vernier caliper. (Fig. 1-4)

FIGURES

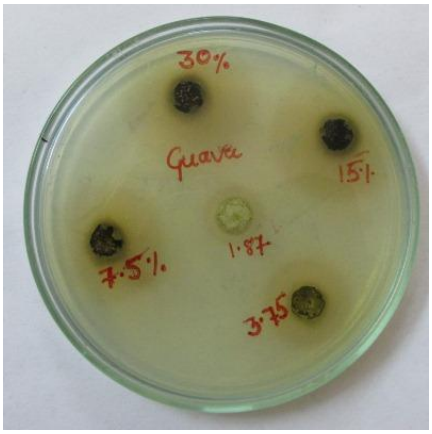

Figure 1 Zone of Inhibition of Various Concentration of ethanolic extract of Guava against S. mutans

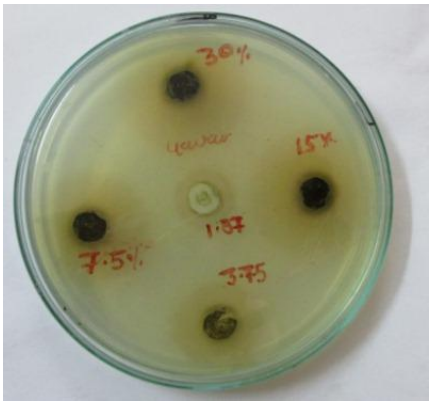

Figure 1 Zone of Inhibition of Various Concentration of ethanolic extract of Guava against E. fecalis

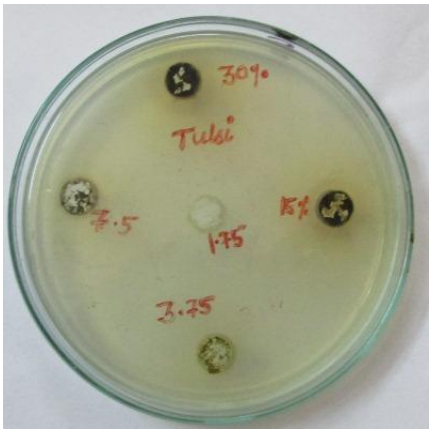

Figure 3 Zone of Inhibition of Various Concentration of ethanolic extract of Tulsi against S. mutans

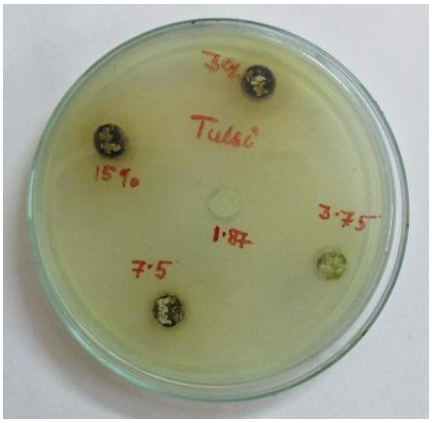

Figure 4 Zone of Inhibition of Various Concentration of ethanolic extract of Tulsi against E. fecalis

RESULT

Otained data were appraised observationally as no statistical tests were required. At the 1.87% concentration, a minimum zone of inhibition of 10 mm, 8mm were seen for 100 µl Guava against S. mutans and E. faecalis respectively. However Tulsi was not effective against S. mutans and E. faecalis at 1.87% concentration. Increasing the concentration further produced a larger zone of inhibition as shown in Table 1. A maximum zone of inhibition of 26 mm was achieved in Guava when compared with Tulsi (16 mm) at the 30% concentration. Similarly, Guava was more effective against S.mutan and E. fecalis than Tulsi at all concentration.

TABLE 1 Zone of Inhibition (mm) of Various Concentration of Ethanolic Extract of Guava and Tulsi against S. mutan and E. fecalis

	Concentrations (%)	**30**	**15**	**7.5**	**3.75**	**1.87**
S. mutans	**Guava**	26	22	16	14	10
	Tulsi	16	12	10	8	00
E.faecalis	**Guava**	20	18	14	10	8

	Tulsi	12	10	8	4	00

DISCUSSION

Injudicious use of antibiotic has led to the development of many resistant strains and also has many adverse effects. Therefore, effective and safer herbal products are needed to be developed against oral infection. The chemical composition of Tulsi and Guava is highly complex, containing many phytochemicals, the proportion of which may vary considerably between strains and even between the plants of the same field. Furthermore, the quantity of many of these constituents is affected by differing in growing, harvesting, processing and storage conditions, which are not yet well understood.[9] Eugenol (l-hydroxy-2-methoxy-4-allylbenzene), the active constituent present in Tulsi, which may be responsible for the therapeutic potential of Tulsi. The other important constituents include ursolic acid and carvacrol. The antimicrobial activity of Tulsi can be attributed to these constituents.[10] Pytochemical studies of Guava has indicated the presence of bioactive compound such as tannin, flavonoids, phenols, terpenoids , essential and fixed oils, alkaloids and reducing sugar. [11] Ethanol was used as a solvent in our study because the phytochemicals in Tulsi and Guava are more soluble in alcohol when compared to distilled water. Normal saline, an inert solvent, was used to dilute the extract to neutralize the effect of alcohol, which itself is an antiseptic, attributing the result solely to Tulsi and Guava.

Various evidences have shown that chlorhexidine was found to effective against S.mutan and E.fecalis. However, the well-known side-effects like staining of teeth and restoration, taste sensation alteration and development of resistant microorganisms, may limit the long-term use of chlorhexidine. In comparison with herbal medicines, Guava and Tulsi are abundantly available, easily accessible, economically feasible and culturally acceptable and may possess no side-effects, hence it can be recommended for long-term use.

CONCLUSIONS

The results of the current study suggest that the extract of Guava and Tulsi can be used as mouthwash, a root canal disinfection or intracanal medicament as they can be effective, economical and free of side effects. However, extensive but a more extensive in vitro and in vivo studies on larger samples are required to evaluate the antimicrobial effect of Guava and Tulsi.

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