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Efficacy of *Psidium guajava* and *Mentha piperita* Fresh Leaves Juice on Perio Pathogens

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ABSTRACT

The research was assessed to evaluate the efficacy of fresh juice of *Psidium guajava* and *Mentha piperita* against selected aerobic and anaerobic bacterial strain. The fresh juice was extracted and evaluated for its antimicrobial activity on anaerobic pathogens like *Fusobacterium nucleatum* (Fn), *Porphyromonas gingivalis* (Pg) and *Prevotella intermedia* (Pi). Antimicrobial activity was evaluated by Disc diffusion method and Minimum inhibitory concentration. The results indicate that *Psidium guajava* and *Mentha piperita* leaves shows significant Antimicrobial activity due to presence of flavonoids.sec.

INTRODUCTION

Long before since from the ancient time, humankind depended on the trees and herbs for medicines to alleviate ailments, search for better health, fragrance, flavours and food. In ancient time human beings depended on animals and plants for his food, shelter demand etc. Over 5000 years peoples of India and China kept on use of plants for the purpose of food as well as to protect from disease. The role of medicinal plants as primary tools in the preservation of health as well as prevention and management of diseases is realised with alarming concern in recent days.[1] Most individuals suffer at some time in their life from localized episodes of disease in the mouth caused by the imbalance in the composition of their natural oral flora.[2] These diseases include dental caries, periodontal diseases and oral candidiasis.[3]Oral hygiene is a great problem in today's world because of improper nutrition, junk food, time interval in between meals, improper brushing and various habits like use of Tobacco chewing, Smoking etc. This all disturb environment of oral cavity which is suitable to develop different oral pathogens. More than 700 different bacterial species have been identified from human oral cavity and the majority of them are associated with dental plaque.[4]

Most of the dental problems are due to microorganism (Oral Pathogens) like Fusobacterium, Actinomyces, Porphyromonas, Prevotella, Eubacterium, Bacteroids, Staphylococcus, Streptococcus, Enterococcus etc. These oral pathogens creates simple to sever periodontal disease, which may leads to various diseases of oral cavity such as Subgingival plaque, Enamel caries and Dental plaque.[5]

Few measures can be followed to avoid periodontal diseases:

1. Brushing properly on a regular basis at least twice a daily.

2. Flossing daily and using interdental brushes.

3. Using soft toothbrush to prevent damage to tooth enamel and sensitive gums.

4. Avoid tobacco chewing and smoking.

5. Avoid junk foods.

6. Regular dental check-up and professional teeth cleaning as required.[6]

The treatment of oral disease includes clinical Treatment like scaling, root planning, synthetic antibiotics therapy for examples Amoxicilline, doxycycline, metronidazole, tetracycline, ciprofloxacin etc.

Drawbacks and Side effects of synthetic drugs:

The treatment for periodontitis is non-surgical scaling which may remove subgingival bacteria but fail to remove pathogenic organisms which are located in the subepithelial gingival tissue.

Microbial debridement may fail to remove pathogenic organism because of their location in subepithelial gingival tissues, crevicular epithelial cells, other anatomic features complicating adequate instrumentation. Moreover, periodontal pathogens frequently colonize oral mucosa, tongue dorsum, tonsils.[7]

Flouride may be used to control caries with efficiency and safety regarding general health or dental side effects such as fluorosis. Flouride available in the ionic form in the oral cavity is able to counterbalance the mineral losses caused by acid production in the biofilm, by inducing the precipitation of the less soluble mineral phase flourapatite in the tooth structure. When available at very low concentrations in the mouth, fluoride can induce the precipitation of minerals on teeth. Long use of fluorides leads to discolouration of teeth with white spots on tooth enamel.[8]

In modern dentifrices the herbal drugs are used like *Acacia nilotica* (babul), *Salvadora persica* (meswak), *Ficus religiosa* (banyan), *Azadirachta indica* (neem), *Malus domestica* (apple), *Glycyrrhiza glabra* (liquorice).[9] Some plants have shown good activity against oral organism and are used in dental disorders they are *Arctium lappa*, Black Cohosh (*Rhizoma Cimicifugae*), Black pepper (*Piper nigrum), Areca nut*.[10]

Psidium cattleianum,[11] *Luffa operculata, Peltophorum pterocarpum,*[12]*Bioaron C,*[13]*Cinnamomum cassia, Allium sativum,*[14] *Cassia fistula Linn, Ficus glomerata Roxb, Ricinus communis Linn.*[15] These are some of the plant and plant products having antimicrobial activity on anaerobic and aerobic pathogens.

Similarly, *Psidium guajava* leaf, fruits and bark extract has been shown antimicrobial activity.[16] Mentha piperita is another plant which is used in food preparations and also has antimicrobial effect.[17]

There are scanty of reports of *Psidium guajava* and *Mentha piperita* fresh leaf juice on Fusobacterium nucleatum (Fn), Porphyromonas gingivalis (Pg), Prevotella intermedia (Pi). So this study is planned to evaluate the antimicrobial efficacy of the fresh leaves juice of *Psidium guajava* and *Mentha piperita***.**

MATERIALS AND METHODS

Collection and authentication of plant materials

In the present study, the matured leaves of *Psidium guajava* belonging to family Myrtaceae and *Mentha piperita* belonging to family Lamiaceae were collected from fields around Belgaum city in the month of April-May and were authenticated by taxonomist Dr. Harsha Hegde and the herbarium has been preserved at regional medicinal centre. The authentication number for *Psidium guajava* and *Mentha piperita* is RMRC-1338 and RMRC-1339 respectively.

Preparation of fresh juice

The fresh leaves were collected from fields of Belgaum and washed thoroughly with water. The leaves then taken grounded to obtain fresh juice. It is stored in a closed vessel for further studies.

Antimicrobial study

The antimicrobial property of *Psidium gaujava* and *Mentha piperita* was done by using following anaerobic oral pathogens like *Fusobacterium nucleatum* (Fn), *Porphyromonas gingivalis* (Pg), *Prevotella intermedia* (Pi).

Disc diffusion Method

Agar plates were brought to room temperature before use. Using a loop, the colonies were transferred to the plates. Visually turbidity adjusted with broth to equal that of a 0.5 McFarland turbidity standard that has been vortexed. Alternatively, standardized the suspension with a photometric device. Within 15 min of adjusting the inoculum to a

McFarland 0.5 turbidity standard, sterile cotton swab was dipped into the inoculum and rotated it against the wall of the tube above the liquid to remove excess inoculum. Entire surface of agar plate was swabbed three times, rotating plates approximately 60ºC between streaking to ensure even distribution. Inoculated plate was allowed to stand for at least 3 minutes but no longer than 15 min before making wells. Hollow tube was taken of 5mm diameter, heated. Pressed it on above inoculated Agar plate and removed it immediately by making a well in the plate. Likewise, six wells prepared on each plate. 75, 50, 25, 10 and 5µl of compound was added into the respective wells on each plate. Plates were incubated within 15 min of compound application. Plates were inverted and stacked them no more than five high. Finally incubated for 24-48 hrs at 37ºC in incubator. Diameter was measured of inhibition zone to nearest whole millimeter by holding the measuring device.

Minimum inhibitory concentration (MIC)

9 dilutions of each drug have to be done with Thioglycollate broth for MIC. In the initial tube, 20 microliter of drug was added into the 380 microliters of Thioglycollate broth. For dilutions, 200 microliters of Thioglycollate broth was added into the next 9 tubes separately. Then from the initial tube, 200 microliters was transferred to the first tube containing 200 microliters of Thioglycollate broth. This was considered as 10^{-1} dilution. From 10^{-1} diluted tube, 200 microliters was transferred to second tube to make 10^{-2} dilution. The serial dilution was repeated up to 10^{-9} dilution for each drug. From the maintained stock cultures of required organisms, 5 microliters was taken and added into 2ml of Thioglycollate broth. In each serially diluted tube, 200 microliter of above culture suspension was added. The tubes were incubated for 24 hours and observed for turbidity.[18]

RESULTS

Both the fresh leaves juice were subjected to qualitative test for the presence of various phytochemical constituents: The *Psidium guajava* leaves juice found presence of Carbohydrates, Proteins, Amino acids, Steroids, Glycosides, Flavonoids, Alkaloids, Tannins and Phenolic compounds where as *Mentha piperita* leaves juice found presence of Carbohydrates, Proteins, Glycosides, Flavonoids and Alkaloids.

The *In vitro* antimicrobial activities of *Psidium guajava* and *Mentha piperita* against anaerobic pathogens and their activity potentials were quantitatively assessed by the presence or absence of inhibition zones, zone diameters and MIC values. In Table 1, *Psidium guajava*

and *Mentha piperita* has shown maximum inhibition zones for Fn, Pg and Pi at 75 µl concentration. *Psidium guajava* and *Mentha piperita* has highest effect on Pg as compare other pathogens. The maximum inhibition of zones against Fn for *Psidium guajava* and *Mentha piperita* juice were 15mm and 11mm respectively. The maximum inhibition of zones against Pg for *Psidium guajava* and *Mentha piperita* juice were 19mm and 15mm respectively. The maximum inhibition of zones against Pi for *Psidium guajava* and *Mentha piperita* juice were 18mm and 14mm respectively.

Table No. 1: Disc diffusion method

Sr. No.	CONCENTRATION OF DRUG	ZONE OF INHIBITION						
		Fusobacterium nucleatum		***Porphyromonas gingivalis***		***Prevotella intermedia***		**Metronidazole**
		Psidium guajava	***Mentha piperita***	***Psidium guajava***	***Mentha piperita***	***Psidium guajava***	***Mentha piperita***	
1	5 µl	04mm	R	06mm	R	05mm	R	S
2	10 µl	05mm	R	10mm	07mm	07mm	05mm	S
3	25 µl	07mm	05mm	13mm	09mm	10mm	08mm	S
4	50 µl	10mm	08mm	15mm	12mm	14mm	12mm	S
5	75 µl	15mm	11mm	19mm	15mm	18mm	14mm	S

In Table 2, the MIC values against Fn for *Psidium guajava* and *Mentha piperita* juice were 3.12 µl and 12.5 µl respectively. The MIC values against Pg for *Psidium guajava* and *Mentha piperita* juice were 3.12µl and 6.25µl respectively. The MIC values against Pi for *Psidium guajava* and *Mentha piperita* juice were 3.12µl and 6.25 µl respectively. This shows *Psidium guajava* juice has better antimicrobial effects on Fn, Pg, and Pi than *Mentha piperita* juice.

Table No. 2: Minimum inhibitory concentration method

Sr. No.	CONCENTRATION OF DRUG	MINIMUM INHIBITORY CONCENTRATION DETERMINATION						
		Fusobacterium nucleatum		*Porphyromonas gingivalis*		*Prevotella intermedia*		Metronidazole
		Psidium guajava	*Mentha piperita*	*Psidium guajava*	*Mentha piperita*	*Psidium guajava*	*Mentha piperita*	
1	0.2 µl	R	R	R	R	R	R	S
2	0.4 µl	R	R	R	R	R	R	S
3	0.8µl	R	R	R	R	R	R	S
4	1.6 µl	R	R	R	R	R	R	S
5	3.12 µl	S	R	S	R	S	R	S
6	6.25 µl	S	R	S	S	S	S	S
7	12.5 µl	S	S	S	S	S	S	S
8	25 µl	S	S	S	S	S	S	S
9	50 µl	S	S	S	S	S	S	S
10	100 µl	S	S	S	S	S	S	S

DISCUSSION

The present study was aimed to explore antimicrobial activity of leaves of *Psidium guajava* and *Mentha piperita.* It is observed that these plants have been used traditionally for its antiulcer activity, antidiabetic activity, anticancer activity anti-inflammatory activity, antioxidant activity etc.

The phytochemical analysis of *Psidium guajava* and *Mentha piperita* leaves revealed the presence of active constituents in *Psidium guajava* leaves juice like carbohydrate, proteins, amino acids, alkaloids, flavonoids, steroids, glycosides and tannins whereas in *Mentha piperita* like carbohydrate, proteins, flavonoids, glycosides and alkaloids.

In the present study, are anaerobic pathogens namely *Fusobacterium nucleatum, Porphyromonas gingivalis* and *Prevotella intermedia* of antimicrobial activity of *Psidium guajava* and *Mentha piperita*.

Antimicrobial agents act selectively on vital microbial functions with minimal effects or without affecting host functions. Different antimicrobial agents act in different ways. Antimicrobial agents may be described as either bacteriostatic or bactericidal. Bacteriostatic antimicrobial agents only inhibit the growth or multiplication of the bacteria giving the immune system of the host time to clear them from the system. Complete elimination of the bacteria in this case therefore is dependent on the competence of the immune system. Bactericidal agents kill the bacteria and therefore with or without a competent immune system of the host, the bacteria will be dead.

Antimicrobial agents can be categorized further based on the structure of the bacteria or the function that is affected by the agents. These include generally the following: Inhibition of the cell wall synthesis, Inhibition of ribosome function, Inhibition of nucleic acid synthesis, Inhibition of folate metabolism, Inhibition of cell membrane function. [19]

Research is still going on to know the constituents responsible for this activity. Reports show that Antimicrobial activity is because of presence of flavonoids, steroids and tannins. The flavonoids are the derivative compound of benzopyrane. These are commonly found in various parts of plants and are rich in leaves. Flavonoids are having antioxidant properties. These flavonoids are reported to possess various pharmacological activities and also have Antibacterial activity. Flavonoids rich plants are used as antimicrobial agents.[20,21]

Psidium guajava and *Mentha piperita* leaves contains coumarins, essential oil, flavonoids, triterpenes and ellagitannins which known to have antimicrobial properties.[22] In the present study, the effect of each drug is seen on anaerobic bacteria which is not evaluated earlier.

However, further studies need to be carried out to confirm the chemical constituents responsible for the antibacterial effect.

CONCLUSION

The present study was aimed to explore the Antimicrobial activity of leaves of *Psidium guajava* and *Mentha piperita* by using perio pathogens.

On phytochemical analysis, fresh leaves juice of *Psidium guajava* confirmed the presence of carbohydrates, proteins, amino acids, steroids, glycosides, flavonoids, alkaloids and tannins.

Whereas leaves juice of *Mentha piperita* carbohydrates, glycosides, flavonoids, alkaloids are confirmed respectively.

In the present study with the referred to the obtained result the fresh juice of *Psidium guajava* leaves remains more antimicrobial activity compared to *Mentha piperita* attributed to the presence of phytoconstituent most like Flavonoids, Alkaloids, Tannins, Amino acids, Glycosides and steroids. All these phytoconstituents already have been reported with Antimicrobial activity.

The present findings indicate that these plants can be used in the treatment of periodontal diseases, further studies need to be carried out to confirm it clinically.

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