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Comparison of Anti-bacterial Activity of Different Concentrations of Betel Leaves *(Piper betle)* and Curry Leaves (*Murraya koenigii*) Extract on *Streptococcus mutans* - An *in vitro* Study

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***Background*:** The nature has answer to cure every disease; medicinal plants are richest bio-resource of traditional systems of medicine. In Ayurvedic medicine the Betel leaves are useful for various systemic diseases. The practice in India is to chew Betel leaves alone or with other spices, which acts as "breath fresheners" and helps in preventing halitosis. Curry leaves are used as taste maker in Indian Cooking.

***Aim*:** To compare antibacterial activity of Betel leaves and Curry leaves extract on *Streptococcus mutans* using different concentrations.

Methodology: Extracts of Curry leaves and Betel leaves were prepared by cold maceration technique using both alcohol as well as aqueous solutions. Different dilutions of distilled water were prepared viz., 20%, 40%, 60%, 80% and 100% of aqueous extracts, while 100% alcohol was used to prepare dilutions of alcoholic extracts. The dilutions of alcoholic extracts were prepared in two concentrations:-50% and 100%. Ditch plate method was used to measure the effects of extracts on growth of *Streptococcus mutans*. Inhibition zones were measured with use of Vernier caliper and compared. Experiment was repeated thrice and means were calculated.

***Results*:** Extracts of curry leaves and betel leaves showed significant antimicrobial activity against *Streptococcus mutans*. It was found that 100% alcoholic extract of Betel leaves is most effective with maximum zone of inhibition while 20% aqueous extract of curry leaves is least effective. *Piper betle* extract has more antibacterial activity than curry leaves extract.

Conclusion: Commonly using Curry leaves and Betel leaves extract can be used to prepare mouthwashes which are effective against cariogenic bacteria. These mouthwashes might acceptable, an easily accessible and provide cheaper alternative to commercially available allopathic mouthwashes leading to achievement of affordable quality health care.

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KEYWORDS
Piper betle, Curry leaves, Streptococcus mutans, Anti-bacterial activity.

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INTRODUCTION

Dental diseases are recognized as major public health problems throughout the world. Various epidemiological studies showed that tooth decay is the most common affliction of mankind. Dental caries is an infectious and transmissible disease and acidogenic bacteria especially *Streptococcus mutans* is associated with this disease in humans[1].

Various synthetic chemical agents have been evaluated over the years with respect to their antimicrobial effects against dental caries. However, most of them are associated with various side effects and are also expensive. Thus, patients are showing their inclination towards natural preparations which are comparatively affordable and have minimal or no side effects. Even now, more than three-fourths of the world's population relies mainly on plants and plant extracts for healthcare [2].

Betel leaves (*Piper betle*) is one of the important plants in the Asiatic region. It ranks second to coffee and tea in terms of daily consumption [2]. Betel leaves (*Piper betle*) are reputed in the Indian Ayurvedic system of medicine for its medicinal properties. The practice in India is to chew the leaves alone or with areca nut and other spices, which act as breath fresheners. It is an aromatic, stimulant herb, with a spicy, clove-like flavour [3].

A study has shown that there is significant relation between paan chewers (Betel quid) and low caries incidence[4].

The Curry leaf (*Murraya koenigii)*, on other hand is grown all over India for its aromatic leaves which is used daily as an ingredient in Indian cuisine. The leaves of *Murraya koenigii* are also used as a herb in Ayurvedic medicine due to their anti-diabetic, anti-oxidant properties [4].

The present study aims to identify, evaluate and introduce simple inexpensive and effective method to prevent and control caries.

AIM

To assess antibacterial activity of Betel leaves and Curry leaves extract on *Streptococcus mutans* using different concentrations.

OBJECTIVES

- To compare the anti-bacterial activity of different concentrations of Betel leaves *(Piper betle*) and Curry leaves (*Murraya koenigii)* aqueous extract on *Streptococcus mutans*.
- To compare the anti-bacterial activity of different concentrations of Betel

leaves *(Piper betle*) and Curry leaves (*Murraya koenigii)* alcoholic (C_2H_5OH) extract on *Streptococcus mutans.*

- To create baseline for production of affordable and accessible oral health care products to prevent dental caries.

MATERIALS AND METHODS

Procurement of Leaves

Betel leaves *(Piper betle*) and Curry leaves (*Murraya koenigii)* were obtained from local market of Belgaum and were dried in sun light. They were grinded to get fine powder.

Preparation of the Extract

Aqueous Extract and alcoholic (C_2H_5OH) extracts were prepared using cold maceration technique[5, 6]. The technique includes steps namely 24- hours maceration as shown in Figure 1. 24 hours maceration was followed by filtration which is shown in Figure 2**.** The filtered solution was kept on Water bath as shown in figure 3. Finally, pure extracts of leaves was obtained which were used to prepare different concentrations of test dilutions as shown in Figure 4.

Preparation of dilutions

Serial dilutions of aqueous extract were prepared using distilled water - 20%, 40%, 60%, 80% and 100% while, 50% and 100% dilutions of alcoholic extract were prepared using 100% ethyl alcohol (C_2H_5OH).

Procurement of the micro-organisms

Freeze dried forms of the microorganisms *Streptococcus mutans (MTCC 497)* was obtained from Microbial type culture collection, Chandigarh.

Preparation of the culture media for the study

The ampoules containing freeze dried forms of the microorganisms were opened and these contents were added to nutrient broth and incubated at 37°C overnight. The growth obtained on the plate was transferred on to a blood agar plate to test the antimicrobial activity[1].

Figure 1 24 hours Maceration

Figure 2 Filtration

Figure 3 Water bath

Figure 4 Pure Extract

Ditch plate method[4, 6]

The agar plates were divided in to various portions. In the centre of each portion ditch was prepared on the agar plates with the help of sterile glass rod. The ditches were filled with 0.5ml drop of extract as shown in Figures 5 and 6**.** The procedure was repeated for different concentrations of Betel and Curry leaves extracts. The plates were then incubated at 37°C for 48 hours in McIntosh-Fildes' anaerobic jar as shown in Figure 7. After which they were examined for the size of the inhibition zones. The zones of Inhibition were clearly seen as shown in Figure 8 and Figure 9.

Measurement of Inhibition Zone

The Vernier callipers was use to check largest diameter of zone of Inhibition. The whole experiment was repeated thrice.

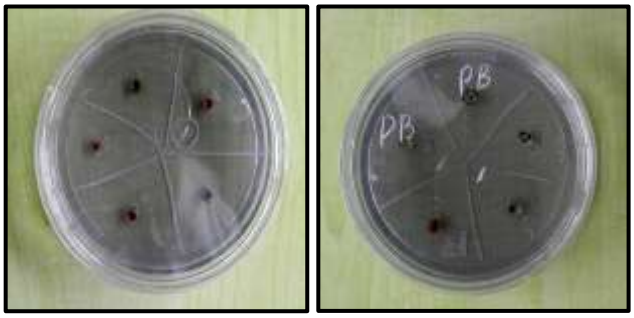


Figure 5 and 6 Ditch Plate method

Zones of Inhibition (Aqueous and Alcoholic)

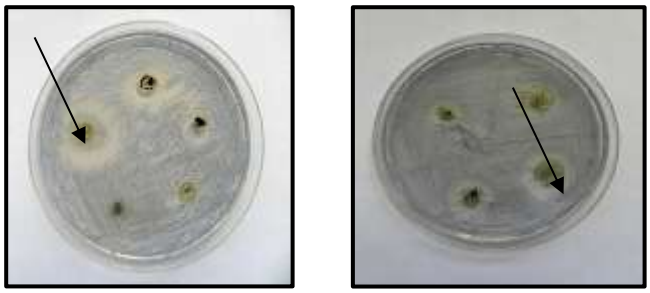


Figure 8 and 9 Zones of Inhibition (Aqueous Alcoholic)

RESULTS

All the results were compiled and entered in the excel sheet (Microsoft Excel 2007). Descriptive Statistics were used to get mean and standard deviation of inhibition zones. Non parametric Mann Whitney U test was used to compare readings from the two groups. The software used was SPSS (Statistical Package for the Social Sciences; Version 17) to do statistical analysis. The 'p' value was set at 0.05.

The effect of various concentrations of Betel leaves and Curry leaves extracts on *Streptococcus mutans* is tabulated in Table1.

Table 1 Readings of Inhibition Zones in millimeters (mm)

Leaves	Sr. no	20% Aqueous	40% Aqueous	60% Aqueous	80% Aqueous	100% Aqueous	50% Alcohol	100% Alcohol
Betel Leaves	1	1.5	2.2	2.6	2.8	3.6	4.3	5.8
	2	1.2	2.2	2.4	2.8	3.9	4.2	6.1
	3	1.4	2.1	2.5	2.9	3.7	4.5	5.7
Curry Leaves	1	0.0	0.0	1.3	1.7	2.1	3.1	3.7
	2	0.0	1.2	1.3	1.8	2.3	3.4	3.9
	3	0.0	1.0	1.4	1.9	2.4	3.1	4.1

Table 2 shows mean zone of Inhibition with aqueous extract concentration while Table 3 shows mean zone of Inhibition with alcoholic extract.

Table 2 Mean Inhibition Zones in millimeters (mm) with aqueous extract concentration

%AQUEOUS			Mean	n	Std. Deviation	p value
20	**Pair 1**	**CL**	0.00	3	0.00	0.037*
		PB	1.36	3	0.15	
40	**Pair 1**	**CL**	0.73	3	0.64	0.046*
		PB	2.16	3	0.05	
60	**Pair 1**	**CL**	1.33	3	0.05	0.046*
		PB	2.51	3	0.10	
80	**Pair 1**	**CL**	1.80	3	0.10	0.046*
		PB	2.83	3	0.05	
100	**Pair 1**	**CL**	2.26	3	0.15	0.050
		PB	3.73	3	0.15	

*Statistically Significant; CL – Curry Leaves; PB – Betel leaves

Comparing 20% aqueous extracts of both leaves; mean zone of inhibition of Betel leaves was (1.36 ± 0.15) while with Curry leaves there was no significant zone of inhibition. This difference was found statistically significant with p= 0.037. (Table 2). Comparing 40% aqueous extracts of both leaves , mean zone of inhibition of Betel leaves extract was (2.16 ±0.05**)** while with Curry leaves extract, it was (1.33 ± 0.05). This difference was found statistically significant with p=0.046. (Table 2)

Comparing 60% aqueous extracts; mean zone of inhibition of Betel leaves extract was (2.51 ± 0.10**)** while with Curry leaves extract, it was (0.73± 0.64). This difference

was found statistically significant with p=0.046. (Table 2)

Comparing 80% aqueous extracts of both leaves; mean zone of inhibition of Betel leaves extract was (2.83 ± 0.05**)** while with Curry leaves extract ,it was (1.80 ± 0.10). The difference among two was found statistically significant with p=0.046. (Table 2).The mean zone of inhibition of Betel leaves extract (100% aqueous) was (2.26 ± 0.15**)** while with Curry leaves extract (100% aqueous), it was (3.73 ± 0.15). The difference among two was found statistically insignificant (p=0.05). (Table 2)

The mean zone of inhibition of Betel leaves (50% alcoholic) extract was (4.33 ± 0.15**)** while with Curry leaves (50% alcoholic) extract, it was (3.20 ± 0.17). The difference among two was found statistically insignificant (p=0.05). (Table 3)

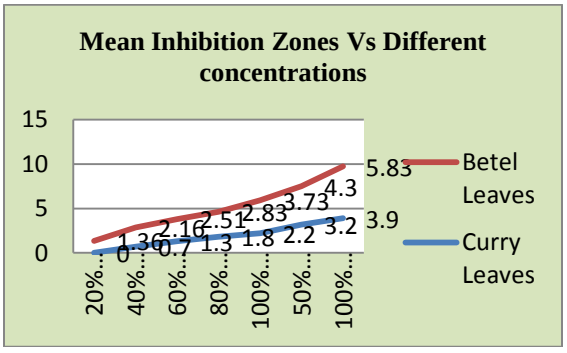
It was found that maximum mean zone of Inhibition was obtained with 100% Betel leaves alcoholic extract which was 6.1mm (5.86 ± 0.2 mm). (Table 1 and 3) There was no statically significant difference between the mean zones of inhibition of 100% alcoholic extracts of both the leaves with (p=0.05). (Table 3)

Table 3 Mean Inhibition Zones with alcoholic extract concentration

ALCOHOLIC %			Mean	n	Std. Deviation	p value
50	Pair 1	CL	3.20	3	0.17	0.050
		PB	4.33	3	0.15	
100	Pair 1	CL	3.90	3	0.20	0.050
		PB	5.86	3	0.20	

*Statistically Significant; CL – Curry Leaves; PB – Betel leaves

Overall comparison of both the groups found that there is a statistically significant difference between the two groups (p=0.005). When a graph was plotted between the mean inhibition zones vs. different concentrations (Graph-1); we found that at 20% aqueous concentrations



Graph 1- Mean Inhibition Zones millimeters (mm) Vs Different concentrations.

there was no significant inhibition zone present. With increase in concentration, there was an increase in the mean zone of inhibition. Highest zone of inhibition was obtained with 100% concentration of the test solution.

DISCUSSION

In the present *in vitro* study it was shown that aqueous as well as alcoholic extracts of Betel and Curry leaves inhibit growth of

Streptococcus mutans. which is main causal factor for Dental caries. Research suggests that Betel leaves contain fatty acids and hydroxychavicol which have antibacterial and antifungal properties[8,9]. The antimicrobial and antifungal activity of Curry leaves is due to presence of essential oils such as thymol, eucalyptus, menthol and terpenes[10].

The alcoholic extract showed higher antimicrobial activity than aqueous extract. It may be because fatty acids are much more active in alcohol compared to water. Additionally, alcohol itself has some antibacterial activity whereas water has none.

CONCLUSION

Further *in vivo* studies are needed to observe the effects of Curry leaves and Betel leaves extracts on salivary pH, dental enamel and other oral soft tissues to enable the preparation of chemical plaque controlling agents like mouth washes. Mouthwashes prepared from herbal, locally available ingredients such as Betel and Curry leaves may be more accessible, economical and acceptable alternatives to conventional therapies, which could make the vision of affordable and quality oral health care, a reality. Because of minimal side effects these remedies can be used to improve oro-dental health in pediatric patients as well as those who are allergic to commercially available chemical plaque controlling agents.

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