ANTIMICROBIAL AND SYNERGISTIC ACTIVITY OF INGREDIENTS OF BETEL QUID ON ORAL AND ENTERIC PATHOGENS

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ABSTRACT

In this study, antimicrobial and synergistic activity of ingredients of betel quid i.e. kattha, lime, betel leaf, betel nut, cardamom, clove and fennel seeds was tested against microbial population of oral cavity and four enteric pathogens namely *Staphylococcus aureus, Salmonella typhi, Escherichia coli and Shigell flexneri*. For this purpose two methods were used. Pour plate method was used for calculating the reduction in microbial population in oral cavity and disk diffusion method was used for detecting the effect of ingredients of betel quid on enteric pathogens. In the first method, microbial population of saliva before and after chewing above ingredients individually and in different combinations was compared. It was found that the each ingredient of betel quid separately and in different combination shows reduction in microbial population by different percentage. The highest percentage of reduction in microbial population was shown by the combination of betel leaf, lime and kattha (Blk) followed by betel leaf, cardamom etc. In the second experiment it was found that the bacteria investigated showed most susceptibility against kattha followed by clove and then combination of betel leaf, lime and kattha. From the study it was concluded that chewing of betel quid after every meal reduces the population of oral micro flora which may be responsible for dental carries and bad breath. It was also seen that enteric pathogens like *Staphylococcus aureus, Salmonella typhi, Escherichia coli and Shigell flexneri* could be inhibited by ingredients of beetle quid.

Key words: Antimicrobial activity, synergistic activity, betel quid, oral pathogens, enteric pathogens.

INTRODUCTION

The practice of chewing the betel (*Piper betel*) for its stimulating qualities is indulged in between a quarter and a tenth of the world's population, which makes it one of the most popular of all psychoactive substances(Norton 1998). In India, Burma, Nepal, Sri Lanka and other parts of South Asia, as well as Southeast Asia, the betel leaves are chewed together in a wrapped package along with the areca nut and mineral slaked lime (calcium hydroxide). Catechu, called "Kattha" in Hindi or "kaath" in Marathi, and other flavoring substances and spices might be added. This combination is known as a "betel quid". In India, betel leaves are used as masticators together with scraped areca nut, lime, cardamom, clove, kattha and fennel seeds.

Dental carries is the most common problem that affects all age groups. Tooth decay occurs when the teeth are frequently exposed to food containing carbohydrates. After taking food there are some bacteria which grow on the teeth and leads to the formation of dental plaque. It is the process in which the enamel and the dentine are demineralized by acids produced by bacterial fermentation of carbohydrates, which is responsible for oral dental carries as well as bad breath. In the prospective of oral health maintenance, the aqueous extract of *Piper betel* has showed positive antiplaque activities that act on dental plaque bacteria at the early phase of plaque formation (Fathilah and Rahim, 2003). Brushing removes some of these microorganisms, but those that persists starts making the plaque again. Betel leaves, areca nut, fennel seeds, clove, cardamom, etc are traditionally chewed as mouth fresheners. Generally these natural mouth fresheners are consumed separately or as a betel quid. So, the present study is an attempt to understand the effect of traditional habitual chewing of betel quid and its ingredients, individually and in different combinations on oral as well as enteric pathogens. It was determined whether, owing to their antimicrobial properties, betel leaves, clove, fennel seeds, cardamom, areca nut, kattha and lime individually or in different combinations are able to inhibit the population of oral microorganisms or not and which combination is best for that.

Similarly effect of the different ingredients of betel quid on enteric pathogens like *Staphylococcus aureus, Salmonella typhi, Escherichia coli and Shigell flexneri* was also determined.

MATERIALS AND METHODS

A) Effect of ingredients of betel leaf on oral microbial population

Collection of plant materials:

Betel leaves, betel nuts, cardamom, clove, and fennel seeds, lime, kattha were procured from the local market.

Collection of saliva sample:

The saliva from individuals before and after masticating two mature betel leaves was collected in two sterile containers. In similar manner samples were collected before and after masticating betel nuts, cardamom, clove buds, fennel seeds individually and in following combinations: Betel leaf, lime, kath(Blk) and betel nut, (Blk) and cardamom, (Blk) and clove, (Blk) and fennel seeds, and finally all combined together(betel quid).

Effect on oral pathogens

All the above collected saliva samples were diluted five times in sterile normal saline and 0.1 ml of each was added in tubes containing sterile molten nutrient agar and then poured into sterile Petri plates. The plate was incubated overnight at 37^0C. The number of colonies was counted and the number of bacteria present was calculated as:

No. of viable bacteria (Cfu/mL) = No. of bacterial colonies × dilution.

B) Antibacterial activity against enteric pathogens:

Preparation of crude aqueous extract

All the plant material was washed carefully with running tap water followed by sterile distilled water, air dried and then pounded using a grinder and stored in air tight bottle. 1 g of each grinded sample was soaked in 10 ml sterile distilled water and left undisturbed for 24 h. after 24 h the mixture was boiled for 30 min. The extract was allowed to cool. The prepared aqueous extract was filtered.

Inoculum preparation

Cultures used for study were *Escherichia coli* (MTCC 729)*, Staphylococcus aureus* (MTCC 96)*, Shigella flexneri* (MTCC 1457)*, and Salmonella typhi* (MTCC 98). For every experiment, freshly prepared sterile nutrient broth (5 ml) was inoculated from the slant and incubated at 37^0C for 24 h. After 24 h incubation loop full of culture was transferred to another freshly prepared sterile nutrient broth and incubated at 37^0C for near about 2 h so as to get the density of 0.5 Mac Farland standard i.e. 10^6 cfu/mL.

Antibacterial assay

Antimicrobial susceptibility test was carried out by Kirby Bauer disc diffusion method. All assays were applied in triplicates and the means were calculated.

Table 1: Reduction in microbial population of oral cavity

Ingredients of Betel Quid	Reduction in microbial population in oral cavity (%)
Betel leaf	57.05
Betel nut	21.08
Cardamom	54.50
Clove	44.48
Fennel seeds	50.00
Blk	59.42
(Blk)+Betel nut	52.58
(Blk)+Cardamom	48.57
(Blk)+Clove	51.47
(Blk)+Fennel seeds	37.96
Betel quid	41.97

RESULTS AND DISCUSSION

In the present study, total 7 ingredients of betel quid were tested for their antimicrobial and synergistic activity on oral and enteric pathogens. The results obtained were as follows.

Antimicrobial and synergistic activity on oral pathogens:

All the ingredients of betel quid showed reduction in microbial population of oral cavity individually and in different combinations as shown in table-1 and fig.-1.

On the basis of statistical analysis of observation it was found that betel leaf, betel nut, the combinations of Blk, Blk and clove and also whole betel quid showed significant reduction in microbial population of mouth cavity.

Antimicrobial and synergistic activity on enteric pathogens:

The aqueous extracts of above ingredients individually and in different combinations were prepared and tested for its antimicrobial activity against *Escherichia coli, Staphylococcus aureus, Salmonella typhi* and *Shigella flexneri* by disc diffusion method. It was observed that the betel nut, kattha, and clove show maximum activity against test organisms. The combination of Blk showed moderate activity while betel leaf, cardamom, fennel seeds, lime and whole betel quid did not show any antimicrobial activity. (Table-2 and figure-2)

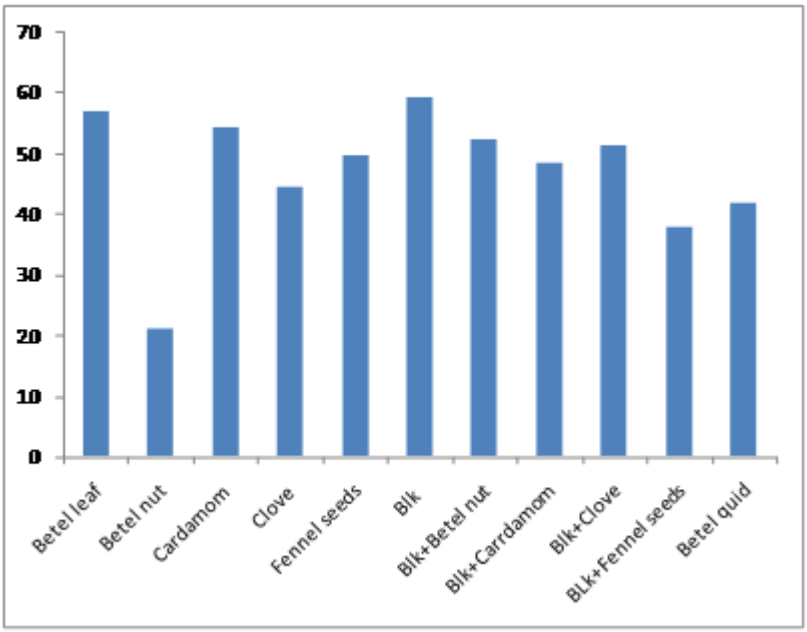

Fig. 1: Reduction in oral microbial population.

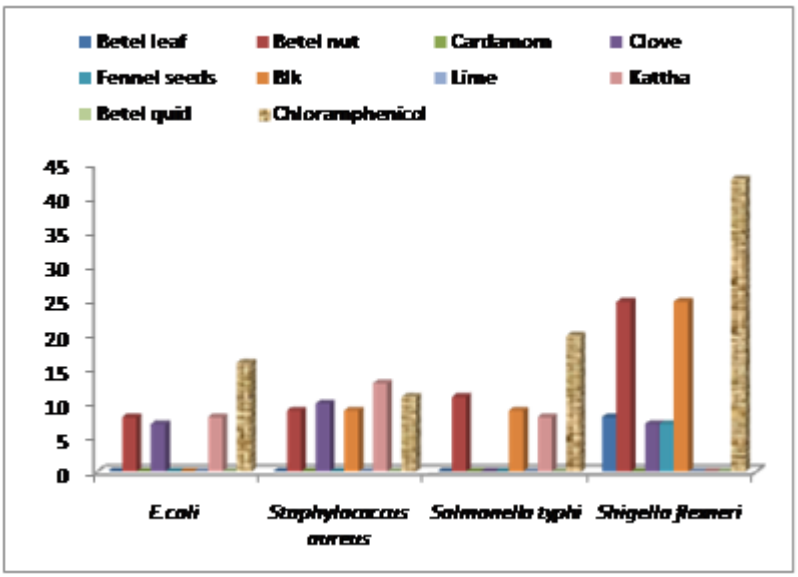

Fig. 2: Antimicrobial activity of constituents against test pathogens.

Table 2- Antimicrobial effect against enteric pathogens.

	Zone of inhibition in mm			
Ingredients of Betel Quid	***E. coli***	***Salmonella typhi***	***Staphylococcus aureus***	***Shigella flexneri***
Betel leaf	0	0	0	8
Betel nut	8	11	9	25
Blk	0	9	9	25
Cardamom	0	0	0	0
Clove	7	0	10	7
Fennel seeds	0	0	0	7
Kattha	8	8	13	0
Lime	0	0	0	0
Betel quid	0	0	0	0
Chloramphenicol	16	20	11	43

Saini *et al.,* in 2009 stated that the microbial count of saliva is more than 1 lakh cfu/ml (colony forming unit). Microbial flora starts decreasing after chewing betel leaf. They observed that betel nut also decrease oral flora to great extent. In this study it was found that the microbial flora of oral cavity (saliva) was decreased by chewing of natural mouth fresheners individually and in different combination in varying degrees. It was found that there was a significant reduction in total microbial count of the mouth after masticating the betel quid. The count was found to be reduced to 59.42% in presence of betel leaf, lime and kattha. Betel leaf, cardamom, Blk and betel nut and Blk and clove reduced the count by more than 50%. Fatilah et al in 2009 in their study also demonstrated bacteriostatic effect of betel leaf on dental plaque bacteria. The effect of betel leaves on oral pathogens was studied by Bissa *et al* (2007). Along with leaves of different landraces, the effect of fruits of cardamom and clove buds was also tested by them. Different combinations, viz. betel leaves and cardamom; betel leaves and clove; betel leaves, cardamom and clove were also applied. All the tested materials gave good results against the oral microbes but the most effective was the combination of betel, cardamom and clove. Gururaj *et al* in their study also demonstrated that cardamom and clove chewing reduce the microbial flora of oral cavity significantly.

In our study, the combination of Blk showed highest reduction in microbial population. As it contains lime it produces alkaline saliva which may inhibit the growth of oral microorganisms. The main constituents of betel leaf are fatty acids, Hydroxyl fatty acids, esters and hydroxyl chavicol (Katsura *et al.,* 2001) which are said to exhibit antimicrobial activity (Pauli, 2002). This combination also showed very good results against *Salmonella typhi, Staphylococcus aureus* and *Shigella flexneri*. It was also seen that betel nut showed highest antimicrobial activity against enteric pathogens. Kattha which is powder of stem of *Acacia catechu* shows surprising inhibition of *E.coli, Staphylococcus aureus* and *Salmonella typhi.* Adeltrudes and Marina, (2010) found that the *Piper betle* oil have significant antibacterial and antifungal activity against *S. aureus, Streptococcus pyogens, Candida albicans* and *Trycophyton mentagrophytes.*

The ingredients of betel quid showed significant reduction in microbial population individually and in different combination, so these can be used as an effective mouth fresheners which can prevents dental carries and bad breath. Among this betel nut, clove, Blk and Kattha also exhibited antimicrobial activity against enteric pathogens. So it can be said that, chewing of betel quid after every meal is good for oral as well as enteric health.

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