Antimicrobial Effect of chewing Tamboolam (betel leaves and its combinations) by testing saliva of volunteers

**ABSTRACT**
Betel chewing is a custom practiced in all religions, which helps in digestion. It is used as medicine to cure Oral ulcers, cancers, periodontal diseases and to control bad breath. Areca nut, cardamom, cloves and lime are added along with it. Each ingredient has its own antimicrobial activity. Areca nut protects against tooth decay and strengthens gums. Clove contains Eugenol, which acts as local anesthetic for tooth aches. Cardamom is used to freshen the breath and supports smooth digestion. In this study, saliva samples were collected from ladies of various age groups before and after mastication of tamboolam and results were compared for different combinations of plates with the help of control set. The microbial load reduction was high in combination of piper betel leaves + cardamom + clove of 90% and in PBL+AN+CL+CA+L piper betel leaves + arecanut + cardamom + clove + lime of 85%.

**KEYWORDS**
Betel chewing, Oral hygiene, bactericidal, combinations of tamboolam

**INTRODUCTION**
Ayurveda is the science of life developed by great Acharyas and seers on the principles of Pancachutas, Saptadhatu and Tridhasa. Any undesired events (in food style, habitat, life style, seasonal, geographical; or accidental factors) cause dramatic changes in the structure of one or more Saptadhatu (Brahmachary, 1982). Ayurveda gives specific treatment methods for diseases related to different dhatus. This dhatu divides Dhatus into two distinct groups. They are ‘Sthiiy or Poshya’ and ‘Asthiy or Poshaka’. Basic tissues are used to strengthen the body by the Sthiiy dhatus. Rasan Kaphha is situated in tongue, taste buds, salivary glands, oral and mouth cavity and is involved in perceiving the taste of food. The Saliva is necessary for protection, lubrication of oral mucosal tissues remineralization of teeth, digestion, taste sensation, stimulation washed out effect, pH balance and phona-ton. It is been used for diagnosis of wide range of diseases due to an easily available, reliable and non invasive diagnostic medium (Mandel, 1990).

The practicing 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 psycho active substances (Norton, 1998). Piper betel leaves (PBL) are used in Chinese folk medicine for the treatment of various disorders like gingivitis, oral ulcers and cancers, dental caries and dental plaque and oral maxillary problems. As per their thought this PBL has the biological capabilities of detoxification, anti oxidation, anti mutation and some extent of antimicrobials (Young and Wang, 2006).

In our traditional Indian customs the practice of taking tamboolam after feast and even after normal food provide complete digestion and act as prophylaxis for digestion also. In North East India betel leaves with a bit of lime and raw betel nut called “Tamul” is consumed by majority of people. In Assam it is a tradition to offer Pan Tamul to guests after tea or meals in brass plates with stand called botta and it have variety of uses during religion and marriage ceremonies. In Assam the boys before one month of marriage are advised to take raw betel nuts with leaves for enhancements of their sexual ability and fertility symbolisms. In south India it is been a tradition to invite guests to Wedding receptions by offering few betel leaves with nuts andrs their blessings are solicited (Jain, 1963). Betel leaf provides an aromatic wrapper; areca nut gives the primary taste of in betel chewing, the lime releases the essence of areca nut and gambir enhances the taste.

The areca nut is also a ‘mangala dhraya’ or auspicious material. Betel nut, with the leaf chewed after meals and exchanged in all religious and symbolic auspicious functions, Hanuman too is associated with the betel leaf with devotional making offerings of garlands made from the leaves. Betel leaf and nut along with a bit of lime is chewed for digestion, as an aphrodisiac, as a lip color enhancer, as a mouth freshener, etc. The leaf is also used in the making of deodorants and ayurvedic medicines. Areca nut: areca nuts are chewed with betel leaf for their effects as a mild stimulant, causing a mild hot sensation in the body and slightly heightened alertness, although the effects vary from person to person. The effect of chewing betel and the nut is relatively mild and could be compared to drinking a cup of coffee.

The US food and Drug Administration do not strictly regulate the herbs and dietary supplements. There is no guarantee of strength, purity or safety of products containing or claiming to contain cloves or clove oil. As per Indian tradition use of Betel leaves and its combinations (tambulam) have no restrictions to consumption. Chewing Areca nut is an increasingly rare custom in the modern world. Yet once, not so long ago, areca nut—taken with the leaves of the betel tree and lime paste—was widely consumed throughout South and South East Asia by people of all social classes, and was considered an essential part of daily life (Bhakuni et al., 1969).

The ubiquitous red—stained lips and blackened teeth associated with betel chewing are sported by one tenth of the human race and one fifth of the global population. Nearly all of them were repelled by it and called betel chewing a “unhygienic, ugly, vile, and disgusting…” habit but lot of traditional studies showed that tamboolam has their own medicinal effects in human practice and their combination provide the wide antimicrobial effect. All these criteria on the backdrop of this present investigation, an attempt has to be made to find out the effect of tamboolas on oral microbes. Tambula, as the betel preparation is called in Sanskrit, is derived from the term tamra meaning copper indicating red colour. This red colour is alluded to because of one of the most popular ingredients of paan namely catechu or katha. According to Sushruta, the patriarch of ancient Indian medicine, paan keeps mouth clean strengthens the voice, tones the teeth and guards against diseases. It is also said to aid in digestion and purify blood. To keep this in mind, the present investigation highlighted the importance of tamboolam on oral flora.

**Materials and Methods**
Preparation of Tamboolam and its combination
Fresh greenish, spicy, betel leaves were brought from the petty shop and brought to the laboratory. Betel leaves are washed with sterile distilled water twice and two leaves were
Mastication of "Tamboolas"

The combinations of tamboolas were given to the selected individuals and they were allowed to masticate. After complete mastication they are requested to swallow and the saliva was collected in aseptic container and transferred to the laboratory for assessment. Control was collected before the mastication of Tamboolas: i.e., the normal oral saliva test sample was collected after mastication of Tamboolas. For oral micro flora assessment, mouth washing was done 2 times with sufficient amount of autoclaved water. This wash served as a stock solution of mouth cavity micro flora. The experiment was started with mastication of betel leaves and its combinations. Two matured leaves after through surface washing were masticated as such in the mouth and the saliva produced was collected in a sterile container.

Processing of Samples

One ml of mouth washing (control) and one ml of saliva sample after masticating the combination were airdried in sterilized Petri dishes to which 20 ml of sterilized, cooled nutrient agar was dispensed. Experiments were also performed by masticating different combinations like Betel leaves, betel leaves + areca nut, betel leaves + lime and betel leaves + areca nut + lime. The Petri plates after processing were incubated at 37°C for 24 to 48 hours.

The bacterial population was measured by counting the number of bacterial colonies from the plates. All the tripel plates of each set were assessed bacteriologically by standard method using colony counter. This experimental protocol was assessed and found to be effective against bacterial population of mouth cavity. All the test sample processed plates were compared with control sets. The reduction of bacterial colonies on betel leaves and its combinations were assessed.

Results and Discussion

The experimental materials included in this study were found to be effective against bacterial population. The detailed reduction rate of Betel leaves and their combinations were approximately estimated by their reduction in colonies compared with control saliva. The detailed descriptions of various age groups are depicted in Figure 1.

Betel Leaves

The saliva obtained after mastication of entire betel leaf reduced the micro flora in pregnant ladies it reduced up to 50 % whereas in non-pregnant girls it approximately reduced up to 50 % but it varies from person to person as compared to control. Some studies proved that Betel leaves of meetha reduced micro flora approximately 56% whereas micro flora reduction of 50% when betel leaves of lanki. Meetha and lanki are two different varieties of betle leaves (Sharad et al., 2007). The chief constituent of the betel leaf is of volatile oil and contains 2 phenol chavativel and chavicol (Burade et al., 2005). The phytochemical investigation of betel leaves showed that it has high amount of tannins (Wiart, 2004). The crude extract of PBL may contain anti carcinogenic activities that are related to decrease acid production and changes to the ultra structure of Streptococcus mutans (Nalina and Rahim, 2007).

BETEL + ARECA MIXTURE

In this combinations the reduction of micro flora was not that much variable from crude betel lead mastication. Approximately 50% reduction was observed in this combination. As per the literature the alterations is saliva flow rate (SFR) and salivary pH are observed in habit AN Chewers. The alteration is dependent on the type of Areca nut chewers. The alteration in SFR and pH are vital in reduction in micro flora. Moreover the complex action of AN chewing is also reflected as variation in SFR and pH (Mandel, 1990; Chu, 2001).

BETEL + ARECA + LIME

This is traditional combination, which is taken after heavy feast or even after daily bowl of rice. This combination shared approximately 55% reduction in all the samples included. Betel leaves + areca nut + lime chewing is a part of many Asian and pacific countries and is often chewed in ceremonies and gatherings.

BETEL + ARECA + LIME chewing is a part of many Asian and pacific countries. The PBL + AN + Lime chewing is a part of many Asian and pacific countries and is often chewed in ceremonies and gatherings. The Preparation techniques and concentration vary from region to region. Usually the Betel leaf was wrapped with grated Areca nut alone with some lime (Calcium oxide or Calcium hydroxide). This combination will provide better extraction of alkaloids from piper betel. The reduction of micro flora using these combinations is depicted in Table 1. The bacterial population that causes dental caries and those causing diarrhea is being affected by chewing Tamboolam and ingestion of its juice respectively especially Streptococcus mutans. The pathogenic bacteria get affected by chewing Tamboolam, which is the positive aspect of this study. Based on these results it is concluded that most remarkable effect on oral microbial population is due to synergistic effect of combination of PBL + AN + L. Spices are known to effect biological effect and have been traditionally used for many disorders. Many herbs and supplements have not been thoroughly tested and effectiveness may not be proven. This investigation was done on tradition linked with scientific theories. Some of these conditions and combinations are potentially serious and should be recognized by a qualified health care provider.

Non-Vegetarian food is regarded as one of the causative agents of diseases. Repeated use of meat and fish causes wound infestation in the stomach and intestine. Uric acid content in the blood increases with increases meat consumptions, which causes joint arthritis and so also heart diseases. Meat proteins are not good for cancer patients. Animals are frightened before they were killed and adrenaline is produced in bulk. Adrenalin infested meat could be a source for many diseases (Chu, 2001). Increased use of meat and eggs increases cholesterol. Alcohol consumption reduces the balance to vitamins assimilation, hyper tension, cerebral palsy, angina, pectoris, breast cancer, etc. Red wine increases HDL cholesterol. This problem of non-vegetarian and alcohol consumption was partly reduced by taking Tamboolas frequently. Most South East Asians chewed this combination from adolescent onwards (custom of taking after marriage), and then virtually without a break until death. For those who indulged Betel areca & lime as important as the daily bowl of rice. Yet once life without betel would have seemed unsupportable to South East Asians as a whole, and its preparation, presentation and consumption were considered a significant social race. Based on the result it is to be concluded the remarkable that the oral microbial population...
due to individual effect and synergistic effect

**Figure 1: Distribution of Age groups in selected samples**

![Distribution of Age groups in selected samples](image)

**Table 1: Reduction of microflora using combinations of Tamboolam**

| Sample | Control | PBL | PBL + AN | PBL + L | PBL + AN + L |
|--------|---------|-----|----------|--------|-------------|
| Tam 01 | 616     | 308 | 247      | 278    | 124         |
| Tam 02 | 415     | 207 | 158      | 108    | 83          |
| Tam 03 | 342     | 171 | 138      | 154    | 69          |
| Tam 04 | 349     | 174 | 142      | 197    | 71          |
| Tam 05 | 420     | 210 | 168      | 189    | 84          |
| Tam 06 | 543     | 271 | 218      | 218    | 109         |
| Tam 07 | 590     | 290 | 234      | 261    | 116         |
| Tam 08 | 516     | 258 | 207      | 232    | 104         |
| Tam 09 | 706     | 353 | 283      | 318    | 141         |
| Tam 10 | 746     | 373 | 299      | 336    | 148         |
| JPT 001| 920     | 462 | 368      | 261    | 92          |
| JPT 002| 278     | 140 | 112      | 89     | 28          |
| JPT 003| 313     | 159 | 127      | 93     | 32          |
| JPT 004| 426     | 217 | 170      | 122    | 43          |
| JPT 005| 486     | 246 | 196      | 138    | 49          |

PBL- Piper beetle leaves; AN-Areca nut; L-Lime

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