9. Singh, S. S., Pandey, S. C., Srivastava, S., Gupta, V. S., Patro, B. and Ghosh, A. C., *Indian J. Pharmacol.*, 2003, **35**, 85–91.
10. Rao, E. V. and Rao, M. V., *Indian J. Pharm. Sci.*, 1981, **43**, 103–106.
11. Murashige, T. and Skoog, F., *Physiol. Plant.*, 1962, **15**, 473–479.
12. Reddy, B. O., Giridhar, P. and Ravishankar, G. A., *Plant Cell Tiss. Org. Cult.*, 2002, **71**, 253–258.
13. Tukey, J. W., *Trans. N.Y. Acad. Sci., Ser. II*, 1953, **16**, 88–97.
14. Thomas, V. and Mehta, A. R., *Plant Cell Culture in Crop Improvement* (eds Sen, S. K. and Giles, K. L.), Plenum Press, New York, 1983, pp. 451–457.
15. Phan, J. J. and Van Staden, J., *Plant Growth Regul.*, 1998, **26**, 155–158.
16. Weatherhead, M. A., Bourdon, L. and Henshaw, G. G., *Z. Pflanzenphysiol.*, 1979, **94**, 399–401.
17. Fridborg, G., Pedersen, M., Landstrom, L. and Erickson, T., *Physiol. Plant.*, 1978, **43**, 104–106.
18. Haberle-Bors, E., *Z Pflanzenphysiol.*, 1980, **99**, 339–342.
19. Chen, Y., Fan, J., Yi, F., Luo, Z. and Fu, Y., *Plant Cell Tiss. Org. Cult.*, 2003, **73**, 75–80.
20. Knight, S. L. and Mithchell, C. A., *HortSci.*, 1987, **22**, 1307–1309.
21. Sengupta, J., Mitra, G. C. and Sharma, A. K., *Plant Cell Tiss. Org. Cult.*, 1984, **3**, 325–331.
22. Ravishankar, G. A. and Venkataraman, L., *The Philippine J. Sci.*, 1988, **117**, 121–129.
23. Hussey, G., *Science Progr.*, 1978, **65**, 185–208.
24. Jones, O. P., In *Plant Biotechnology* (eds Mantrell, S. H. and Smith, H.), Cambridge University Press, London, 1983, pp. 139–159.
25. Boxus, P., *Acta Hortic.*, 1976, **66**, 35–38.

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Traditions in oral hygiene: Chewing of betel (*Piper betle* L.) leaves

The practice of chewing the betel (*Piper betle*) 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[1]. It is used in an area stretching from east Africa to Polynesia. In India, betel leaves are used as a masticatory (the taste being warm, aromatic and bitter), together with scraped arecanut and lime. The present study is an attempt to understand the effect of traditional *Piper betle* leaves on oral microorganisms. Along with leaves of different betel landraces, the effect of fruits of cardamom and clove buds was also tested. 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.

The betel plant is indigenous throughout the Indian Malay region[2]. The plant is a climber and is trained on poles or trellis in a hot but shady situation. Betel leaf is a necessary ingredient in most Hindu functions and festivals. It is customarily chewed with lime (*choonam*) and arecanut. A study showed that this habit was responsible for preventing osteoporosis[3] in a group of economically, and socially disadvantaged, people. It was inferred that the calcium in the slaked lime was easily assimilated in the presence of betel juice. Betel leaf is aromatic, a carminative and a stimulant. It is also an aphrodisiac and an antiseptic. It improves digestion, clears the voice and cures flatulence. The leaf juice is given internally to treat cough and indigestion in children. The leaves are also used as a counter-irritant to suppress the secretion of milk in mammary abscesses and also have wound-healing property[4]. The oil is an active local stimulant used in the treatment of respiratory catarrhs as a local application or gargle, also an inhalant in diphtheria[5]. The various piper species have been found to have broad spectrum of antibacterial activity[6]. Cardamom (*Elettaria cardamomum*) seeds have a warm, slightly pungent and highly aromatic flavour. The cardamom oil is a precious ingredient in food preparations, perfumery, health foods, medicine and beverages. A good portion is consumed for chewing or as a masticatory item. In medicine, it is used as powerful aromatic, stimulant, carminative, stomachic and diuretic, but rarely used alone. It also checks nausea and vomiting, helps in combating digestive ailments. This spice can be used to freshen breath and support smooth digestion. Clove is a small, reddish brown flower bud of the tropical evergreen tree *Syzygium aromaticum*. Strong in aroma, hot and pungent in taste, cloves are also used as a flavouring agent in food. Cloves are strongly pungent due to an aromatic oil containing eugenol and are also a local anesthetic for toothaches. It is also a strong antiseptic and preservative. Role of *P. betle* in oral hygiene was indicated in several ancient texts[7] and has also been shown in some recent studies[8,9]. In the present investigation it was tested that whether, owing to their medicinal properties, betel leaves, cardamom and clove, individually or in different combinations, were able to inhibit the population of oral microorganisms. In addition, whole preparation of traditional *Pan* (betel leaf, lime, catechu, *Gulkand*, cardamom and clove) was also tested for its effect against oral microbes.

For oral microflora, mouth washing was done three times with sufficient amount of autoclaved water. This wash served as stock solution of mouth cavity microflora for further studies. The experiment was started with betel leaves of two landraces, i.e. *Meetha* and *Lanki*. Two mature leaves after thorough surface washings were masticated as such in the mouth and the saliva produced was collected in a sterilized conical flask. One ml of mouth washing and one ml of this

saliva solution were added to each of a set of three sterilized petri dishes to which 15 ml of sterilized cooled nutrient agar was added. Similar sets were prepared using saliva produced as a result of masticating fruits of cardamom as well as clove buds. For further experiments betel leaves of landrace *Meetha* were used. Experiments were also performed by masticating different combinations, viz. betel leaves and cardamom; betel leaves and clove; betel leaves, cardamom and clove. Finally, sets were also prepared using saliva produced by masticating whole preparation of traditional *Pan* comprising of betel leaf, lime, catechu, *Gulkand*, cardamom and clove. The normal saliva, after washing, was used for preparing the control sets. The petri dishes were incubated at 37°C for 48 h. The bacterial population was measured by counting the number of bacterial colonies on the agar plates.

Figure 1 shows that all the experimental material were found to be effective against bacterial population of mouth cavity. The saliva obtained after mastication of entire betel leaf (*Meetha*) reduced the microflora, approximately 56% as compared to control whereas the microflora reduction was 50%, when betel leaves of *Lanki* was used. The chief constituent of the leaves is a volatile oil varying in the leaves from different countries and known as betel oil. It contains two phenols, betel-phenol (chavibetol) and chavicol[10]. Cadinene has also been found. The best oil is a clear yellow colour obtained from the fresh leaves. The phytochemical investigations of betel leaves showed that it had high amount of tannins[6].

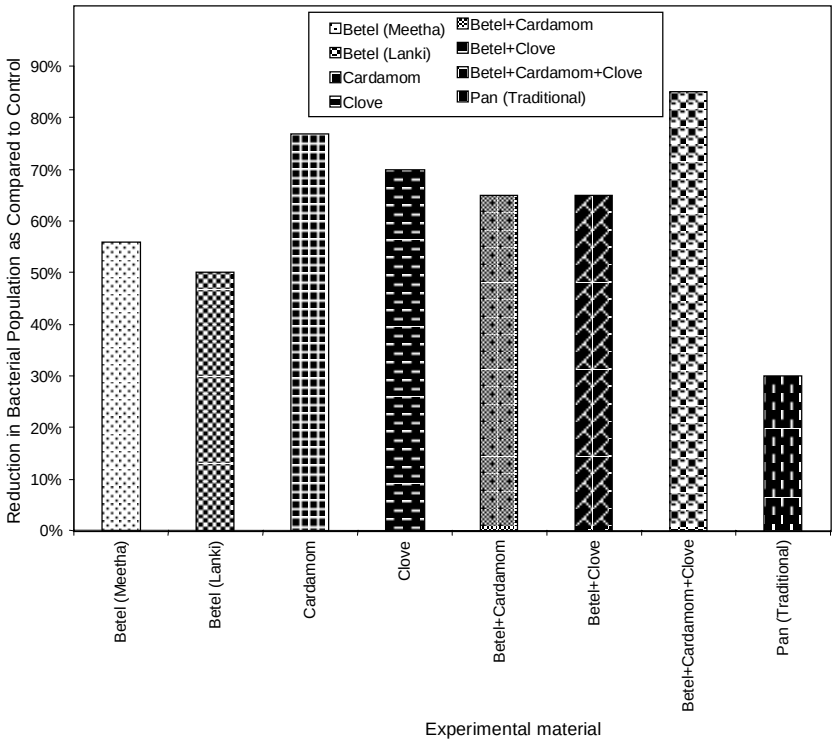

Figure 1. Effect of different extracts on oral bacterial population.

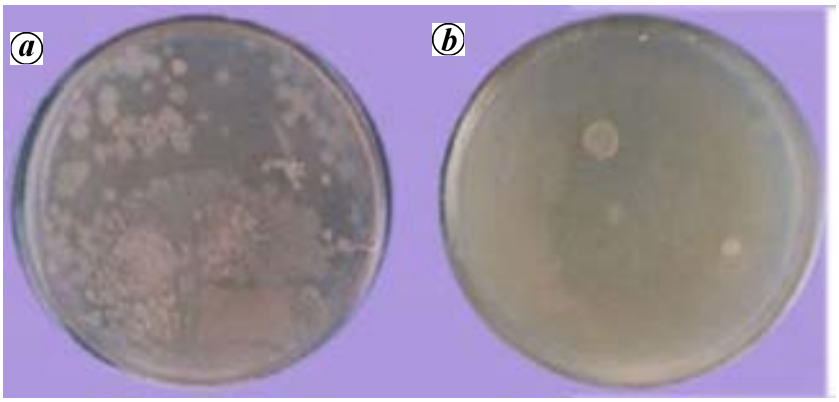

Figure 2. ***a***, Control set; ***b***, Effect of combination of betel leaf, cardamom fruit and clove bud on oral microbes.

The cardamom also significantly inhibited the growth of oral microflora and the reduction was up to 77% as compared to control. The antimicrobial activity of cardamom is usually attributed to the volatile oils present in the seeds[11]. Tiwari and Charya[12] studied the effect of *Elettaria cardamomum* on oral microbial population *in vitro* and concluded that it was some constituents in the pericarp that might be largely responsible for antimicrobial activity of cardamom. Daswani and Bohra[13] have also reported the suppression of *Salmonella typhi* by aqueous extracts of dried pericarps of *Elettaria cardamomum.*

Similarly, clove buds also suppressed the oral microorganisms to 70% and it is evident from the fact that toothpastes contain clove oil as their major constituent. Briozzo *et al.*[14] reported that essential oil of clove, dispersed in concentrated sugar solution had marked germicidal effect against various bacteria. Kalemba and Kunicka[15] reported that essential oils of spices and herbs were found to possess strongest antimicrobial properties. Different combinations were also applied on oral microbes and found to give good results. Both combinations, i.e. betel leaf and cardamom as well as betel leaf and clove buds inhibited the growth of oral microbe population by 65%, but the most marked effect on the oral bacterial population was observed in the saliva obtained after mastication of betel leaf along with cardamom and clove. The combined effect of all the three constituents reduced oral microbe population by 85% as compared to control (Figure 2 *a* and *b*). At last, the effect of whole traditional *Pan* comprising of betel leaf, lime, catechu, *Gulkand*, cardamom and clove was also tested on oral microorganisms, reducing the growth to 30%.

Based on these results it is concluded that most remarkable effect on oral microbial population is due to synergistic

effect of the combination of betel leaf, cardamom and clove. Spices are known to affect biological functions and have been traditionally used for many disorders[16].

1. Norton, S. A., *J. Am. Acad. Demt.*, 1998, **38**, 81–88.
2. Chopra, R. N., Nayar, S. L. and Chopra, I. C., *Glossary of Indian Medicinal Plants*, NISC, New Delhi, 1996, p. 195.
3. http://www.hinduonnet.com/mag/2002/09/08/stories/2002090800470700.htm
4. Santhanam, G. and Nagarajan, S., *Fitoterapia*, 1990, **61**, 458–459.
5. Prajapathi, N. D., *Agro's Dictionary of Medicinal Plants*, Agrobios, Jodhpur, 2003, p. 401.
6. Wiart, C., *Phytotherapy Res.*, 2004, **18**, 783–784.
7. Kumar, N., *Indian J. Hist. Sci.*, 1999, **33**, 19–32.
8. Kumar, N. and Tripathi, R., *Plant Peroxidase News Lett.*, 2000, **15**, 45–48.
9. Ramji, N., Iyer, R. and Chandrasekaran, S. J., *Ethanopharmocology*, 2002, **83**, 149–152.
10. Burade, K. B., Chopade, A. R., Mhasde, M. S. and Nalawade, R. S., *J. Microbial World*, 2005, **7**, 294–296.
11. Karkanthimatham, V. S., Prasath, D. and Rao, G., *J. Med. Aromatic Plant Sci.*, 2000, 683.
12. Tiwari, A. and Charya, M. U., *Nat. Acad. Sci. Lett.*, 2004, **28**, 107–108.
13. Daswani, L. and Bohra, A., *Adv. Plant Sci.*, 2003, **16**, 87.
14. Briozzo, J., Nunej, L., Chirife, J., Herszage, L. and D'Aquino, M., *J. Appl. Bacteriol.*, 1989, **66**, 69–75.
15. Kalemba, D. and Kunicka, A., *Curr. Med. Chem.*, 2003, **10**, 813–829.
16. Ridley, H. R., *Spices*, Macmillan and Co, London, 1983.

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