International Journal of Food, Agriculture and Veterinary Sciences ISSN: 2277-209X (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jfav.htm
2011 Vol.1 (1) September-December, pp.88-91/Zombade et al.
Research Article

EFFECT OF FRESH LEAF JUICE OF *PIPER BETLE* ON *STREPTOCOCCUS MUTANS* AND *CANDIDA ALBICANS*

K.A. Zombade[1], K.A. Gajare [1,2] and A.A. Deshmukh[1,3*]
[1]*Department of Zoology, Govt. Vidarbha Institute of Science and humanities, Amravati*
[2]*Department of Zoology, The New College, Kolhapur*
[3]*Department of Zoology, Shivaji University, Kolhapur*
**Author for Correspondence: ashish_cellbio@rediffmail.com*

ABSTRACT
The increasing prevalence of antibiotic resistance is an alarming situation. There is a need to search for new antimicrobials. Microbes extraordinarily acquire mechanisms to combat the synthetic antimicrobials. Fortunately, the ancient system of Indian herbal medicine; Ayurveda has illustrated a wide range of herbal medicines for different pathophysiological disorders. *Piper betle* is a plant, it's leaves are consumed in India as Pan or betel quid. This plant has various medicinal properties. It is used as a mouth freshener as well as to improve bad breath. We have demonstrated the bacteriostatic activity of *Piper betle* on *E.coli* and *B.subtilis.* In the present investigations attempts are made to study antimicrobial activity of fresh leaf juice of *Piper betle* against two normal inhabitants of human oral cavity 1) *Streptococcus mutans* a causative agent of dental caries and 2) a fungus *Candida albicans* a causative agent of oral thrush. The study was carried out by disc diffusion method, agar well diffusion method and determining the Minimum Inhibitory concentration. For *S. mutans*, there was 13.29 ± 1.5mm zone of inhibition by disc diffusion method and 7.8 ± 0.8 mm by agar well diffusion method. After 24 hours of incubation the zone of inhibition was disappeared by growth of bacteria, indicating the bacteriostatic activity of fresh leaf juice of betel leaf against *S. mutans.* There was no zone of inhibition in *Candida albicans* suggesting no any effect.

Key Words: *Piper betle, antimicrobial activity, Streptococcus mutans, Candida albicans*

INTRODUCTION
The increasing prevalence of multi drug resistant strains of bacteria and occurrence of newly evolved strains having low susceptibility to antibiotics result in life threatening conditions. There is need to search and develop all together different ways and means to overcome these challenges (Zy *et al.,* 2005 Rojas *et al.,* 2006). Plant based antimicrobials may help to endeavor this goal. *Piper betle* is a stout glabrous climber with heart shaped leaves. It is one of the well known medicinal plants used in the treatment of various ailments. Several medicinal values of *Piper betle* are well documented such as anticarcinogenic (Padma *et al.,*1989), as contraceptive (Sarkar, 2000), antioxidant action (Choudhary and Kale 2002, Lei *et al.,* 2003), larvicidal (Wardhana *et al.,* 2007). *Piper betle* is among the plants that have been used for the control of caries and periodontal diseases and bad breath (Ponglux *et al.,* 1987). Razak and Rahim (2003), Fathilah *et al.*(2006) reported the antibacterial activity of crude aqueous extract of leaves of *Piper betle* against *Streptococcus mitis, Streptococcus sanguis* and *Actinomyces viscous*. These are the early colonizers of dental plaque. Bissa *et al.,* (2007) found the remarkable effect on oral microbial population due to the synergistic effect of the combination of betel leaf, cardamom and clove. We have demonstrated the bacteriostatic action of fresh leaf juice of Piper betle on *E.coli* and *B.subtilis* but no any effect on *S. aureus* (Zombade *et al.,* 2011). In the present investigations the antimicrobial activity of *Piper betle* is studied on *Strptococcus mutans* and *Candida albicans*. *Strptococcus mutans* is a normal inhabitant of oral cavity and involved in dental caries and tooth decay. *Candida albicans* is a fungus involved in oral thrush, skin infections and genital tract infections. Therefore, in the present study *Candida albicans* was used to assess antifungal activity of *Piper betle* if any.

MATERIAL AND METHODS

Test organisms: Streptococcus mutans (MTCC 890) and *Candida albicans* (MTTC 183) were procured from IMTECH Chandigarh. *Streptococcus mutans* (MTCC 890) was suspended in brain heart infusion broth and incubated at 37^{o}C for 48 hrs. Thereafter a loopful suspension from this broth was inoculated on slant of brain heart infusion agar for further use.

Candida albicans (MTTC 183) was suspended in Potato dextrose broth and incubated at 37^{o}C for 48 hrs. There after a loopful suspension from this broth was inoculated on slant of Potato dextrose agar and incubated at 37^{o}C for 24 hours. For the antibacterial study the cells from logarithmic phase were used.

Antimicrobial study: Antimicrobial activity was studied by disc diffusion method and agar well diffusion method.

Disc diffusion method (Bauer *et al.*,1966**):** The Whatman filter paper No.1 discs of the diameter 5mm were used. The discs were sterilized in dry air oven. Each disc was soaked in 5µl of fresh leaf juice. Antimicrobial or antibiotic impregnated disc releases the antimicrobial agent into the surrounding medium when placed on the surface of solid agar. A zone of inhibition surrounding the test disc indicates antimicrobial activity. 0.1ml of the inoculum of test organism was spread using sterile glass spreader on the surface of bain heart infusion agar (for *S. mutans*) and potato dextrose agar (for *C. albicans*). A disc soaked in leaf juice was placed on the medium at the center of the plate. The plates were initially transferred to refrigerator for 30 minutes to allow the diffusion of the contents from disc to the surface of the agar and then incubated at 37°C for 12 hrs. The diameter of the zone of inhibition was measured. Disc soaked in Ciprofloxacin (10µg/ml) and clotrimazole (1%) were used as control for antibacterial and antifungal action respectively, whereas discs soaked in sterile water were used as negative control.

Agar well diffusion method (Perez *et al.*,1990)**:** Wells of the diameter of 3 mm were punched in the agar using sterile gel puncher and filled with 10 µl of the fresh leaf juice of *Piper betle.* The antibiotic Ciprofloxacin (10µg/ml) was used as the positive control for antibacterial action and clotrimazole (1%) for antifungal action, while sterile water was used as the negative control. The plates were incubated at 4°C for 30 min to allow diffusion of the contents of the well into the medium and then incubated at 37°C for 12 hours. The diameter of the zone of inhibition was measured.

Determination of minimum inhibitory concentration: It was carried out as per the recommendations of NCCLS (1997).

RESULTS AND DISCUSSION

For *S. mutans* there was a zone of inhibition of 32.29 ± 0.95 mm in control i.e. surrounding the ciprofloxacin disc, whereas in experimental group i.e. *Piper betle* leaf juice soaked disc it was 13.29 ± 1.50 mm. In agar well diffusion method, the zone of inhibition was 34.83 $\pm$ 1.58 mm in ciproflozacin containiong well and 7.8 $\pm$ 0.8mm in Piper betle leaf juice containing well (Figure 1). For *Candida albicans* surrounding the disc and well containing fresh leaf juice of *Piper betle*, there was no zone of inhibition. While surrounding the disc and well containing Clotrimazole the zone of inhibition was 28.5 $\pm$ 1.4 and 28.2 ± 1.2 mm respectively (Figure 2). The minimum inhibitory concentration (MIC) for *S. mutans* was 1.25%.

Zone of inhibition of *S. mutans* surrounding the disc and well of fresh leaf juice of *Piper betle* indicates growth inhibiting property. Nalina and Razim (2006) demonstrated the antiviruence property of fresh leaf juice of *Piper betle* against *S. mutans*. The zone of inhibition surrounding the well containing fresh leaf juice of *Piper betle* was smaller than that of the disc. In the agar well diffusion method, the juice diffuses through the agar while in the disc diffusion method, it diffuses and spreads on the surface of the agar. Therefore the inoculated cells making contact with the leaf juice might have been inhibited to grow. The components in the leaf juice that inhibits the growth of *S. mutans* might be volatile and unstable, therefore till the juice gets diffused through the agar might get inactivated or their concentration might get depleted. This decreased concentration might not be sufficient to inhibit the growth.

As far as the plant derived antimicrobials are concerned, their quantity in the crude juice is very negligible

due to the presence of 90% water in it. Among these antimicrobials most of them are volatile and very unstable in nature, therefore the component that is inhibitory to *C. albicans* till diffused through the well it might get inactivated. Another reason for such type of observation is *C. albicans* has developed resistance to most of the antifungal drugs (Mishra *et al.*,2007). Therefore it might have developed defense to tolerate the unfavourable conditions.

Table 1: Diameter of Zone of Inhibition in mm observed in Disc Diffusion Method and Agar Well Diffusion Method (Incubation Period 12 Hours, Temperature 37°C)

microorganism	Diameter of zone of inhibition in Control in mm (Ciprofloxacin/Clotrimazole)		Diameter of Diameter of zone of inhibition in Experimental group in mm	
	Disc diffusion	Agar well diffusion	Disc diffusion	Agar well diffusion
S. mutans	32.29 ± 0.95	34.83 ± 1.58	13.29 ± 1.5	7.8 ± 0.8
C. albicans	28.5 ± 1.4	28.2 ± 1.2	--	--

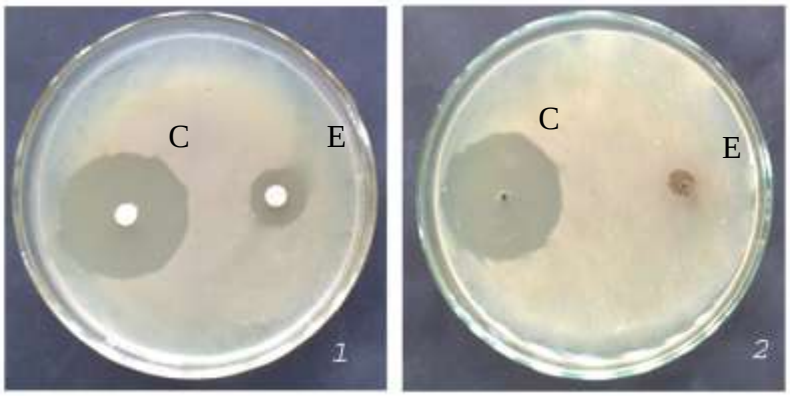

Figure 1: showing the zone of inhibitions observed in *Streptococcus mutans* by disc diffusion method and agar well diffusion method : Control (Ciprofloxacin), E : experimental (Leaf juice of *Piper betle*)

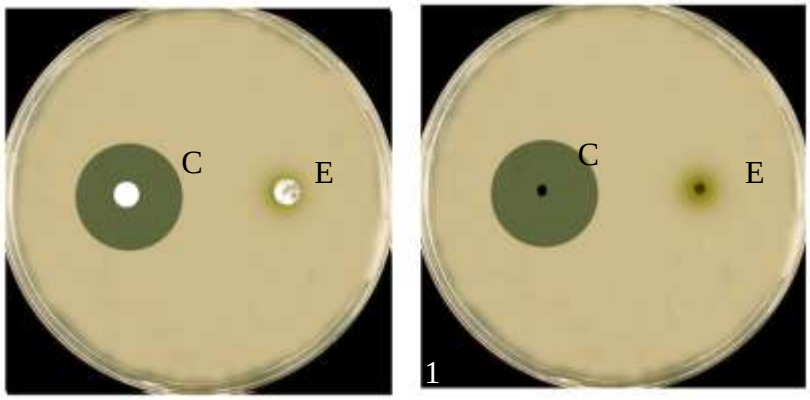

Figure 2: showing the zone of inhibitions observed in *Candida albicans* by disc diffusion method and agar well diffusion method : Control (Clotrimazole), E : experimental (Leaf juice of *Piper betle*)

REFERENCES

Bauer AW, Kirby WM, Sherris JC and Turk M (1966). Antibiotic susceptibility testing by a standardized disk diffusion method. *American Journal of Clinical Pathology* **45**(4) 493-496.

Bissa S, Songara D, and Bohra A (2007). Traditions in oral hygiene: Chewing of betel *Piper betle* L.) leaves *Current Science* **92**(1) 26-28

Choudhury D and Kale RK (2002). Antioxidant and non-toxic properties of *Piper betle* leaf extract: In vitro and in vivo studies. *Phytotherapy Research* **16** 461-466.

Fathilah AR, Bakri MM and Rahim ZHA (2006). The effect of *Piper betle* and *Psidium guajava* extracts on the cell-surface hydrophobicity of selected early settlers of dental plaque. *Journal of Oral Science* **48**(2) 71-75.

Lei D, Chan CP, Wang YJ, Wang TM, Lin BR, Huang CH, Lee JJ and Chen HM (2003). Antioxidative and Antiplatelet Effects of Aqueous Inflorescence *Piper betle* extract. *Journal of Agricultural Food Chemistry* **51**(7) 2083–2088.

Mishra NN, Prasad T, Sharma N, Payasi A, Prasad R, Gupta DK and Singh R. (2007). Pathogenicity and drug resistance in *Candida albicans* and other yeast species a review. *Acta Microbiologica et Immunologica Hungarica* **54**(3) 201-235.

Nalina T and Razim ZHA (2006). Effect of *Piper betle* L. leaf extract on the virulence activity of *Streptococcus mutans* an in vitro study. *Pakistan Journal of Biological Sciences* **9**(8) 1470-1475.

NCCLS specialty collection : susceptibility testing (1997) Wayne, PA : NCCLS.

Padma PR, Lalitha VS, Amonkar AJ and Bhide SV(1989). Anticarcinogenic effect of betel leaf extract against tobacco carcinogens. *Cancer Letters.* **45**(3) 195-202.

Perez C, Paul M and Bazerque P (1990). Antibiotic assay by agar well diffusion method. *Acta Biologiae et Medicinae Experimentalis* **15** 113-115.

Ponglux D, Wong S, Phadungcharoen T, Ruangrungsri N and Likhitwitaya K(1987). Medicinal Plants, *Victory Power Point Corporation, Bangkok*, 194-209.

Razak FA and Rahim ZHA(2003). The antiadherence effect of *Piper betle* L. and *Psidium guajava* extracts on the adhesion of early settlers in dental plaque to saliva-coated glass surfaces. *Journal of Oral Science* **45** 201-206.

Rojas JJ, Ochoa VJ, Ocampo SA and Munoz JF (2006). Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: A possible alternative in the treatment of non-nosocomial infections. *BMC Complementary and Alternative Medicine* **6** 2.

Sarkar M. (2000). The reversible antifertility effect of *Piper betle* Linn. on Swiss albino male mice. *Contraception* **62**(5) 271-274.

Wardhana AH, Kumarasinghe SW, Arawwawala L and Arambewela LS (2007). Larvicidal efficacy of essential oil of betel leaf (*Piper betle*) on the larvae of the old World screwworm fly, Chrysomya bezziana in vitro. *Indian Journal of Dermatology* **52** 43-47.

Zombade KA, Gajare KA and Deshmukh AA (2011). Antibacterial activity of fresh leaf juice of *Piper betle* against *Escherichia coli, Bacillus subtilis* and *Staphylococcus aureus. Indian Journal of Fundamental and Applied Life Sciences* **1**(4) 391-396.

Zy EA, Area A and Aam K (2005). Antimicrobial activity of some medicinal plant extracts in Palestine. *Pakistan Journal of Medical Sciences* **21** 187-193.