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In-vitro antimicrobial activity of *Psidium guajava* (L.) against some isolated oral pathogens

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Abstract

Methanolic extract of *Psidium guajava* stem was investigated for its antimicrobial activities against five isolated oral pathogen. Among the 50 sample collected from patients 26.25% were identified as *Staphylococcus* spp., 25% as *Micrococcus* spp., 23.75% *Streptococcus* spp, 15% *Corynebacterium* spp and 10% Proteus spp.The strongest antimicrobial activity was observed against *Micrococcus spp.*

Keywords:- *Psidium guajava, Antimicrobial*

Introduction

In recent year antibiotic resistance to common antibiotic has been increasing day by day. The mechanism of resistance is either emergence of bacteria or mutation of one or more genes. This situation, the undesirable side effect of certain antibiotic and the emergence of previously uncommon infections has forced scientist to look for new antimicrobial substance from medicinal plant and other sources (Marchese and Shito, 2001). The screening of plant extracts and plant products for antimicrobial activity has shown that plants represent a potential source of new antimicrobial agents (Amani et al., 1998). Recent interest in chewing sticks and their extract has focused on their effects on organisms that are involved in oral infection. Dental caries and periodontal disease i.e. gingivitis and periodontitis are the most common chronic oral diseases worldwide. Although these diseases have affected human beings since prehistorical times, the prevalence of these diseases has greatly increased in modern times. The prevalence of dental diseases in India is approximately 60-65% (Shouri, 1941) while the prevalence of periodontal disease is approximately 65-100% (Kanal et *al.* 1971). Dental caries is an infectious microbial disease that results in localized dissolution and destruction of the calcified tissues of teeth (Ross *et al.,* 1994). Most of the investigators believe that development of caries of enamel is preceded by the formation of microbial plaque in the tooth (Gibbons *et al.* 1963). The human oral cavity is habitat for about 500 cultivable and non cultivable bacterial species (Paster *et al.*2001) up to 100 species can be present in a particular oral cavity (Consensus, 1996) while the majority of these species are commensals, a subset is opportunistic pathogens. They have also been implicated in the etiology of a number of systemic diseases like infective endocarditis, (Barrau *et al.* 2004) respiratory infections, (Mojon *et al.* 2003) cardiovascular diseases, (Okuda et *al.,* 2004) and brain abscess (Corson *et al.* 2001).Oral bacterial isolates resistant to penicillin, metronidazole, tetracycline and macrolides have been reported by researchers from different countries (Sweeney *et al.,*

2004). Such resistant bacteria have also been isolated from infections at extra oral sites (Doern *et al.* 1996). To cope with this problem of antibiotic resistance, a number of strategies such as reduced antibiotic use and antibiotic alternatives have been proposed (Hamilton *et al.,* 2004). Among antibiotic alternatives are therapies derived from complementary and alternative medicine. In fact, there are an overwhelming number of studies on the antibacterial activities of plant and natural products derivatives. Recently some plant extracts have been shown to have activity against resistant bacterial strains (Gibbons *et al.* 2003).

Psidium guajava (L.) (Guava) belong to the family Myrtaceae, grows nearly throughout the country up to 1500 m. it is cultivated commercially in almost all states. The total estimated area being about 50,000 hectares. The important guava growing states are: U.P. Bihar, Uttaranchal. Maharastra, Assam, West Bengal, Andhra Pradesh & Chennai. About half of the total area is reported in Uttar Pradesh. The plant of guava is found almost every garden of Indians. Guava is rich in tannins, phenols, triterpenes, flavonoids, essential oils, saponins, carotenoids, lectins, vitamins, fiber and fatty acids. Guava fruit is higher in vitamin C than citrus (80 mg of vitamin C in 100g of fruit) and contains appreciable amounts of vitamin A as well. Guava fruits are also a good source of pectin – a dietary fiber. The leaves of guava are rich in flavonoids, in particular, quercetin. Much of guava's therapeutic activity is attributed to these flavonoids. The flavonoids have demonstrated antibacterial activity. Quercetin is thought to contribute to the anti-diarrhea effect of guava; it is able to relax intestinal smooth muscle and inhibit bowel contractions. In addition, other flavonoids and triterpenes in guava leaves show antispasmodic activity. Guava also has antioxidant properties which are attributed to the polyphenols found in the leaves (Arima *et al.,* 2002). Bark and leaf extracts have shown to have in vitro toxic action against numerous bacteria. In several studies guava showed significant antibacterial activity against such common diarrhea causing bacteria as *Staphylococcus, Shigella, Salmonella, Bacillus, E. coli, Clostridium, and Pseudomonas* (Abdelrahim *et al.* 2002). The present study was undertaken to investigate the in vitro antibacterial activity of methanolic extract of twigs of *P. guajava* against oral pathogens.

Materials and Method

Collection of sample for isolation of oral pathogen

Total 50 samples were collected from Shree "Mahant Indresh hospital" Patel Nagar, Dehradun. The samples from dentine of teeth were taken into a sterile cotton swab. The cotton swab was placed in each approximal site and then passed along the gingival margin into the next approximal site of both the upper and lower teeth.

Enrichment of the sample

The cotton swabs were enriched in lactose broth (HiMedia) and Trypticase soy broth (HiMedia) for 12 hours at 37^0 C in the incubator. Then the enriched sample was streaked on both general Nutrient Agar and Selective media Brain Heart Infusion Blood Agar (HiMedia), Mac Conkey Agar (HiMedia), Mannitol Salt Agar (HiMedia), and Vogel Johnson Agar (HiMedia).

Isolation and identification of pathogens

The pathogens associated with dental caries, gum and periodontal diseases were isolated from infected tissues and identified by standard morphological and biochemical tests on the surface of the growth of the isolates (Holt. *et al.,* 1994).

Collection of Plant Material

The Plant used were the air dried stem of *P. guajava* (Guava) collected from Forest Research Institute (FRI) Dehradun and Identified by Botanical survey of India, Dehradun.

Extraction of Plant Materials

The air dried and powdered material was extracted, with methanol by soxhlet apparatus by removing the solvent with rotary evaporator (Butchi Type) crude extracts were obtained.

Antibacterial Assay

Preparation of Inoculum

The ideal inoculum after overnight incubation gives the even semi confluent growth. Too heavy inoculum may reduce the size of inhibition zone by many antimicrobial agents from plant source. Using a straight wire touch 5-10 well isolated colonies of particular microorganism against which antimicrobial activity to be tested. Inoculate on the Nutrient Broth Medium. Incubate at 35-37°C for 4 – 6 hour. The density of the inoculums is adjusted to 10^8 cfu/ml by comparing with that of 0.5 Mc Farland Standard.

Agar Well Diffusion

0.1 ml of the original cultures (about10^6-10^7 cells) were added into sterile duplicate sets of Petri dishes and 25 ml of the molten (45° C) Mueller Hinton Agar (HiMedia, Ltd) were poured into Petri dishes. The methanol extract (0.1ml) were placed in wells (8mm diameter) cut in the agar media and plates were incubated at 37° C in the case of bacteria and at 30°C in the case of yeasts (Kývanç et al., 1986). The resulting inhibition zones obtained with bacteria were recorded after 24 hour.

Results and Discussion

The present study was conducted to investigate the antimicrobial activity methanolic extract of *P. guajava* against some isolated oral microorganism. Among the 50 sample collected from patients 26.25% were identified as Staphylococcus spp., 25% as Micrococcus spp., 23.75% *Streptococcus* spp, 15% *Corynebacterium* spp and 10% proteus spp. (Fig.1). Among the 40 microbial samples collected from adult patients, 10.63% were identified as *Staph. aureus*, 4.32% as *Strep. mutans*, 6.38% *Strep. faecalis*, 2.12% *Strep. pyogenis*, 5.25% *Lactobacillus acidophilus*, 2.1% *Ps. aeruginosa* and 4.3% as Candida albicans (Al-Bayati and Sulaiman, 2008).

On the other hand, the methanolic extract of *P. guajava* showed activity against the entire isolated microorganism. Zone of inhibition ranged from 12.82±1.15 - 16.93±1.28 mm. Maximum activity was shown against Micrococcus sp. i.e. 16.93±1.28mm and less active as compared to tested microorganism is against Proteus sp i.e. 12.82±1.15mm. Standard drug Amoxicillin and Doxycycline was active against the entire test microorganism. The extracts of *P. guajava* compared with the standard antibiotics (Doxycycline and Amoxicillin). The plant extracts and antibiotics show almost equal antibacterial activity against oral pathogens. Previous studies reported that the methanolic extract of *Psidium guajava* was also shown to possess antibacterial effect on *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* (Abdelrahim *et al.,* 2002). Gnan and demello (1999) reported a complete inhibition of growth of *S. aureus*, *S. epidermidis* and *Salmonella typhimurium* caused by aqueous guava leaf extract at a concentration of 8 mg/ml. Veeira et al (2001) reported the microbicidal affect of guava sprout

extract (ethanol, acetone and water) upon toxigenic S.aureus and E. coli, performed using radial diffusion. Earlier pharmacological investigation indicated that its fruit and leaves posses antibacterial, hypoglycemic, anti-inflammatory , analgesic, antipyretic, spasmolytic and CNS depressant activities (Begum et al., 2002). Jairaj et al., (1999) also reported the water, alcohol and chloroform extracts of leaves were effective against *Aeromonas hydrophila*, *Shigella* spp., *Vibrio* spp., *Staphylococcus* spp., *Sarcina lutea* and *Mycobacterium phlei*. It can be concluded that the methanolic extract are excellent oral hygiene agents and their use should be promoted based on scientific knowledge of their benefit and proper use. Because it is widely available in India and is inexpensive. *P. guajava* chewing sticks can be great help in developing countries with financial constrains and poor or limited oral health care facilities.

Table-1: Effect of methanolic extract against isolated microorganism

		Zone of inhibition (In mm)		
Microorganism	**No. of isolates**	**MeOH***	**Amoxicillin**	**Doxycycline**
Micrococcus sp.	20	16.93±1.28	17.97±1.25	18.07±1.21
Cornebacterium sp.	12	15.81±1.22	16.67±1.54	16.05±1.62
Staphylococcus sp.	21	15.23±1.14	16.21±1.58	15.72±1.61
Streptococcus sp.	20	16.48±0.97	17.32±1.58	16.47±1.55
Proteus sp.	8	12.82±1.15	14.0±1.38	14.15±1.71

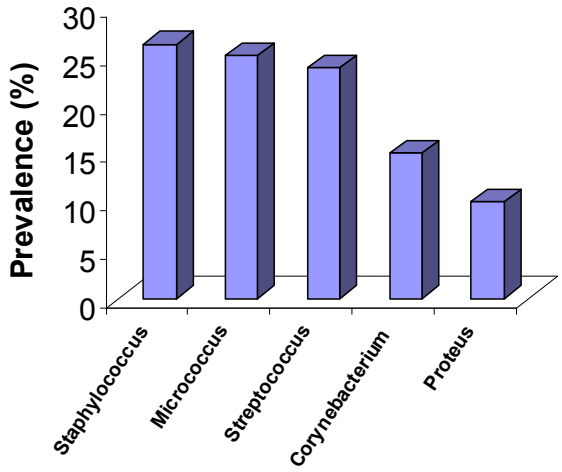

Fig.1: Prevalence of isolated microorganism.

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