

Full Length Research Paper

Antimicrobial activity of propolis against *Streptococcus mutans*

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Accepted 28 June, 2010

The ethanol extract of propolis (EEP) obtained from beehives of honeybee (*Apis mellifera*) was investigated for its antimicrobial activities against *Streptococcus mutans* isolated from dental caries. Agar well diffusion and minimum inhibitory concentration (MIC) determinations were the methods used in this study. The carious tooth was swabbed with a sterile cotton wool and immediately streaked on tryptic soy agar, incubated at 37°C for 48 - 72 h. *S. mutans* was characterized by standard cultural, morphological and biochemical methods. Several dilutions of EEP were made (0.5 - 32 µg/ml), while water and ethanol were used as controls. The EEP at concentrations of 4, 8, 16 and 32 µg/ml showed strong antimicrobial activity against *S. mutans* with inhibition zones of 10 ± 4, 12 ± 4, 20 ± 2 and 24 ± 2 mm, respectively. There were medium to maximum growth of *S. mutans* in the controls of ethanol and water. The results demonstrate that the ethanol extract of propolis has a strong antimicrobial activity and suggest that it may be useful in the treatment of dental caries caused majorly by *S. mutans*.

Key words: Dental caries, antimicrobial activity, *Apis mellifera*, *Streptococcus mutans*, ethanol extract of propolis.

INTRODUCTION

The tooth surface is unique in that it is the only body part that is not subjected to metabolic turnover. It is however subjected to various infections due to factors that favour microbial growth. One of the diseases of the teeth is dental caries (Marsh, 1995). Unless satisfactory treatment is given, dental caries can lead to pain, infection, tooth loss and in severe cases death (Keyes, 1998). An estimated 90% of school children worldwide and most adults have experienced dental caries with the disease being most prevalent in Asian and Latin American countries and least prevalent in African countries (Miller, 1993). Dental caries has been attributed to a number of factors such as age, civilization, dietary habits, race and geographical location (Rippa, 1990; Burt, 1994).

In Nigeria, Adekoya et al. (2006) reported a prevalence of 13.9% in 402 school children in Ile-Ife, Umehi-Koleoso et al. (2006) reported 23.8% in school children in Lagos, Nigeria while Okeigbemen (2004) reported 33% in children

in Egor local govt. area, Edo state. Bacteria associated with dental caries have been reported to include *Streptococcus mutans* and *Actinomyces*. These organisms act on the available carbohydrate in the mouth, breaking it down with the subsequent production of lactic acid which eventually eats up the teeth enamel. *S. mutans* is the leading cause of dental caries worldwide and is considered to be the most cariogenic among the oral streptococci. The relationship between the number of *S. mutans* in dental plaque and the occurrence of dental carriers has been reported (Ikeda et al., 1973).

The use of natural agents against selected oral pathogens has long been reported. Ceanothic acid and ceanothric acid have been shown to inhibit *S. mutans*, *Actinomyces rescosus* and *Phyromonas ginvalis* (Li et al., 1997). Chewing sticks from *Garcinia kola* and *Fagara xanthoxyloids* have also been reported to have antimicrobial activity against oral pathogens (Abimbola and Tolulope, 2006). Propolis, a natural product from honeybee (*Apis mellifera*) has been shown to exert *in vitro* antibacterial action against a number of oral microorganisms and inhibits cell adhesion as well as water

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Table 1. Prevalence of bacteria isolated from dental caries.

Organisms	Prevalence (%)
<i>S. mutans</i>	82
<i>S. sobrinus</i>	46
<i>Lactobacillus</i> sp.	21
<i>S. aureus</i>	14

insoluble glucan formation (Koo et al., 2000).

Although a lot of studies in Nigeria have shown the aetiologic agents/prevalence of dental caries and the antimicrobial activities of some natural and synthetic compounds to these agents, information concerning the antimicrobial activity of ethanol extract of propolis (EEP) on *S. mutans* is depleted in Benin City, Nigeria.

Therefore, this study investigated the antimicrobial activity of EEP on *S. mutans* isolated from a patient diagnosed to have dental caries attending the Central Hospital Benin City, Nigeria. The results of this study may further strengthen the recommendation for the use of ethnomedicine in the treatment and control of microbial infections.

MATERIALS AND METHODS

Isolation of *S. mutans*

The test organism was isolated directly from the tooth of a patient diagnosed with dental caries by a physician. The patient was attending the Dental Clinic of the Central Hospital, Benin City, Nigeria. The infected area of the tooth was swabbed three times with sterile cotton wool to remove debris and saliva. The tooth was then swabbed with another sterile cotton wool and immediately streaked on tryptic soy agar (TSA, Difco, Detroit, MI USA) incubated at 37°C 48 - 72 h.

S. mutans was identified using cultural, morphological and biochemical characteristics as described (Cheesbrough, 2000; Buchanan and Gibbon, 1974). Characteristic colonies were picked from the plates and purified by repeated subculturing. Pure colonies were streaked on nutrient agar slopes in McCartney bottles as stock cultures for antimicrobial activity.

Extraction of propolis

Propolis was obtained from a honey bee market located in Oghara, a community in Delta State, Nigeria. The propolis was collected from beehives and stored at -4°C before use. Thirty gram (30 g) of propolis was ground and homogenized prior to beginning of extraction (Popova et al., 2005). The methods of Trusheva et al. (2007) and Silva et al. (2007) were used for EEP. Two gram of ground propolis was extracted by 25 ml of 70% (v/v) ethanol by orbital shaking at 150 rpm at 25°C for 48 h. The ethanol extract was then filtered through a Whatman No. 42 filter paper and diluted to 100 ml with 70% in a volumetric flask. Various concentrations of 0.5, 1, 2, 4, 8, 16 and 32 µg/ml of EEP solution were made.

Antimicrobial activity of propolis on *S. mutans*

The antimicrobial effect was established if propolis had inhibitory

properties on *S. mutans*. The method of Yang et al. (2007) was used. Briefly, *S. mutans* colonies were transferred into TSB (Difco, Detroit, MI, USA) in a test tube, incubated at 37°C for 12 h to have organisms in the exponential phase of growth. To examine the effect of EEP concentration, the method of Abimbola and Tolulope (2006) was used.

The test organism was sub cultured three times in fresh TSB media to obtain a more vigorous population. One milliliter of the culture was aseptically transferred into sterile petri dishes and 15 ml of molten nutrient agar (Biotec Lab.Ltd,Uk) was poured into the same plate. This was allowed to gel and dry. A sterile cork borer of 5 mm in diameter was used to punch holes in the agar creating a well/ditch. To each well was added different concentrations of EEP.

All plates were labeled and allowed for 2 h for proper diffusion of the extract before incubation at 37°C for 24 h. The mean zones of inhibition was measured and recorded to the nearest mm. A mean inhibition zone greater than 2 mm in three trials was used as the minimum threshold (Akande and Hayashi, 1998). The mean of each trials and the standard deviation was calculated (Ogbeibu, 2005).

RESULTS

The bacteria isolated in this study included *S. mutans*, *Streptococcus sobrinus*, *Lactobacillus* sp. and *Staphylococcus aureus*. *S. mutans* was used in this study because of its high prevalence of isolation as shown in Table 1. *S. mutans* had a prevalence of 82%, while *S. aureus* had 14%. Table 2 shows the inhibitory properties of ethanol extract of propolis. There was no growth of *S. mutans* in EEP concentrations of 4, 8, 16 and 32 µg/ml. It was however resistant to EEP at concentrations of 0.5, 1 and 2 µg/ml as there was growth in the petri dishes. The ethanol and water acted as controls. There was medium growth in ethanol because of its bacteriostatic and bactericidal activities while the growth in water was maximum.

The sensitivity of *S. mutans* to EEP at different concentrations is shown in Table 3. The highest MIC of 32 µg/ml had a zone of inhibition of 24 ± 4 mm while the least was 6 ± 2 µg/ml for a MIC of 2 µg/ml.

DISCUSSION

It is well documented that the bacteria of the genera *Streptococcus*, *Lactobacillus*, *Fusobacterium*, *Corynebacterium* and *Staphylococcus* are normal flora of the mouth and can as well cause dental caries (Rowe et al., 1996; Slots, 1977; Park et al., 1998; Yabao, 2005; Adekoya et al., 2006; Leistevuo et al., 2000). In this study, *S. mutans* was the most prevalent organism (82%). It has been widely reported to be the major organism involved in dental caries due to its ability to create an acidic environment by the breakdown of carbohydrate. The result presented in Table 1 confirms earlier reports (Leistevuo et al., 2000; Uzel et al., 2005; Yang et al., 2007). They reported a high prevalence of *S. mutans* in dental caries.

The antimicrobial activity of propolis against *S. mutans*

Table 2. Inhibitory properties of ethanol extract of propolis (EEP) on *S. mutans*.

Parameters	Propolis concentrations µg/ml						
	0.5	1.0	2.0	4.0	8.0	16.0	32.0
Ethanol extract of propolis	+++	++	++	-	-	-	-
Ethanol	+++	+++	+++	++	++	++	++
Water	+++	+++	+++	+++	+++	+++	+++

Table 3. Minimum inhibitory concentration (MIC) of EEP to *S. mutans*.

Organism	Zone of inhibition (mm)	MIC (µg/ml)
<i>S. mutans</i>	6 ± 2	2
	10 ± 4	4
	12 ± 4	8
	20 ± 2	16
	24 ± 4	32

Mean ± standard deviation.

and other oral pathogens have been reported (Yang et al., 2007; Uzel et al., 2005). Although resistance of some gram negative bacteria to propolis has been reported (Kujumgier et al., 1999; Moreno et al., 1999), the result in Table 2 confirms the antimicrobial activity of propolis to *S. mutans*. It has been reported that the antimicrobial activity of propolis is as a result of the high content of flavonoids present (Trusheva et al., 2007; Silva et al., 2007)

Dental caries is one of the commonest oral diseases in children. This could be due to inadequate brushing time, ineffective brushing technique or both factors (Adekoya et al., 2006). Determination of the antimicrobial activity of propolis will help to improve treatment and control of dental caries.

Conclusion

Apart from the recommended use of fluoride toothpaste and some natural antimicrobial agents, the use of propolis for the treatment and control of dental caries is highly recommended. Also, dental health education and caries preventive programme will minimize caries in children and even in adults.

ACKNOWLEDGEMENTS

We thank the patient who participated in this study and the Staff and Management of Central Hospital, Benin City, Nigeria for their cooperation. Also, special thanks to the honeybee products farmers in Oghara, Delta State Nigeria.

REFERENCES

- Abimbola AO, Tolulope O (2006). Antibacterial effects of thre selected chewing sticks extracts on *Lactobacillus* sp. Int. J. Trop. Med. 1(3): 103-106.
- Adekoya-Sofowora CA, Nasir WO, Oginni AO, Taiwo M (2006). Dental caries in 12 yr old suburban Nigeria school children. Afr. Health Sci. 6(3): 145-150.
- Akande JA, Hayashi Y (1998). Potency of extract contents from selected tropical chewing sticks against *Staph. auricularis*. World J. Microbiol. 14: 235-238.
- Buchanan RE, Gibbon ME (1974). Bergey's Manual of Determinative Bacteriology (8th ed) Williams and Wilkins Baltimore, USA, pp. 450-860.
- Burt B (1994). Trends in caries prevalence in North America children. Int. Dent. J. 44: 403-413.
- Cheesbrough M (2000). District Laboratory Practice in Tropical country (PT 2). University Press, Cambridge, pp. 157-255.
- Ikeda T, Sandham HJ, Bradley EL (1973). Changes in *Strept. mutans* and *Lactobacilli* in plaque in relation to the initiation of dental caries in Negro children. Arch. Oral. Biol. 18: 555-566.
- Keyes PHC (1998). Present and future methods for dental caries control. J. Am. Coll. Dent. 111: 243-249.
- Koo H, Rosalen PL, Cury JA, Ambrosano GMB, Murata RM, Yatsuda R, Ikegaki M, Alencar SM, Park YK (2000). Effect of a new variety of *Apis mellifera* propolis on mutans Streptococci. Curr. Microbiol. 41: 192-196.
- Kujumgier A, Tsvetkova I, Serkedjieva Y, Bamkova V, Christov R, Popov S (1999). Antimicrobial, antifungal and antiviral activity of propolis of different geographic origin. J. Ethnopharmacol. 64: 235-240.
- Leistevuo J, Jarvinen H, Osterblad M, Leistevuo T, Houvinen O, Tenovuo J (2000). Resistance to mercury and antimicrobial agents in *Strept. mutans* isolates from human subjects in relation to exposure to dental amalgam fillings. Antimicrobial. Agents Chemother. 44(2): 456-457.
- Li XC, Cai L, Wu CDC (1997). Antimicrobial compounds from *Ceanothus americanus* against oral pathogens. Phytochemistry, 46: 97-102.
- Marsh PD (1995). The role of microbiology in models of dental caries. Adv. Dent. Res. 2(3): 242-250.

- Miller WD (1993). Agency of microorganisms in decay of human teeth. *Dental Cosmos*. 251: 1-12.
- Moreno MIN, Isla MI, Cudmani NG, Vattuone MA, Sampietro AR (1999). Screening of antibacterial activity of Amaicha del valle (Tucuman, Argentina) propolis. *J. Ethnopharmacol.* 68: 97-102.
- Ogbeibu AE (2005). *Biostatistics: A practical Approach to Research and Data Handling*. Mindex Publishing Co. Ltd. Benin City pp. 73-127.
- Okeigbemen SA (2004). The prevalence of dental caries among 12-15 yr old school children in Nigeria: a report of a local survey and campaign. *Oral Health Prev. Dent.* 2(1): 27-31.
- Park YK, Koo MH, Abreu JSA, Ikegali M, Cury JA, Rosalen PL (1998). Antimicrobial activity of propolis on oral microorganisms. *Curr. Microbiol.* 36(1): 24-28.
- Popova M, Silici S, Kaftanoghu O, Bankova V (2005). Antibacterial activity of Turkish propolis and its qualitative and quantitative chemical composition. *Phytochemistry*, 12: 221-228
- Rippa LW (1990). Has the decline in caries prevalence reduced the need for fissure sealant in UK. A review: *J. Paediatric. Dent.* 6: 79-83.
- Rowe NH, Aron SM, Clark DC, Gume KE (1996). Effect of age, sex, race and economic statute on dental caries experience of the permanent dentition. *Paediatrics*, 57: 457-461.
- Silva BB, Rosalen PL, Cury JA, Ikgaki M, Souza V, Esteres A, Alencar SM (2007). Chemical composition and botanical origin of red propolis, a new type of Brazilian propolis. *Evidence-Based compl. Alt. Med.* 5(3): 313-316.
- Slots J (1977). The predominant cultivable microflora of advanced pseriodontitis. *Scand. J. Dental Caries. R.* 85: 114-121.
- Trusheva B, Trunkova D, Bankova V (2007). Different extraction methods of biologically active components from propolis: a preliminary study. *Chem. Cent J.* 1: 13-17.
- Umeh-Kpoloeso DC, ayanbadejo OP, Oremosu OA (2006). Dental caries prevalence among adolescents in Lagos, Nigeria. 5th Annual Scientific Congress of IADR, Lagos, pp. 6-24.
- Uzel A, Sorkun K, Oncag O, Cogulu D, Gencay O, Salih B (2005). Chemical compositions and antimicrobial activities of four different Anatonian propolis samples. *Microbiol. Res.* 160(2): 189-195.
- Yabao RN, Duante CA, Valendria FV, Lucas M, Kassu A, Nakamori M, Yamamoto S (2005). Prevalence of dental caries and sugar consumption among 6-12 yr old school children in LaTrinidad, Benguet. *Eur. J. Clin. Nutr.* 59: 1429-1436.
- Yang HY, Ho WL, Chang CM, Chou CC (2007). Antibacterial activity of propolis ethanol extract against *Streptococcus mutans* as influenced by concentration, temperature, pH and cell age. *J. Food Drug Anal.* 15(1): 75-81.