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Full Length Research Paper

Antimicrobial activity of propolis extract on bacteria isolated from nasopharynx of patients with upper respiratory tract infection admitted to Central Hospital, Benin City, Nigeria

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The study was aimed at determining the bacterial agents of the upper respiratory tract infection (URTI) and the susceptibility of isolates to propolis. Propolis extract was obtained by 70% ethanol and serial dilutions of 0.25, 0.5, 1, 2, 4, 8 and 10 µg/ml prepared. A total of 250 throat swabs were obtained from patients (age between 15 - 30 years) which were diagnosed with upper respiratory tract infection attending the central hospital, Benin City. Samples were collected between February and December, 2008 from 142 (56.8%) males and 108 (43.2%) females, inoculated on blood agar, eosin methylene blue agar and chocolate agar and incubated at 37°C for 24 - 48 h aerobically except for chocolate agar which was incubated microaerophically. The isolates were characterized by standard microbiological procedures. Of the 250 samples, 160 (64%) had positive cultures with *Haemophilus influenzae* having the highest prevalence (20.8%), followed by *Klebsiella pneumoniae* (19.2%), *Streptococcus pneumoniae* (12.0%), *Moraxella catarrhalis* (10%), *Streptococcus pyogenes* (2%). The highest rate of isolates was from the age group of 15 - 18 years (91). This was significantly higher than other groups p > 0.05. *M. catarrhalis* and *S. pyogenes* were not isolated in age group 23 – 26. propolis antimicrobial activity revealed that all isolates were sensitive to propolis at all concentrations with *K. pneumoniae* and *S. pneumonia* having zones of inhibition of 32 and 30 mm respectively. The findings suggest that propolis is a very effective antimicrobial agent for the treatment and management of URTI caused by bacterial species.

Key words: Upper respiratory tract infection, minimum inhibitory concentration, propolis, bacteria, antimicrobial activity.

INTRODUCTION

An upper respiratory tract infection (URTI) is a non-specific term used to describe acute infections involving the nose, paranasal sinuses, pharynx, larynx, trachea and bronchi (Mossad, 2008). URTIs such as sore throat, ear ache, laryngitis, common cold, otitis media and sinusitis are the most frequently occurred infections of all human diseases and among the leading cause of health services worldwide and have been frequently documented (Huston et al., 1999; Brunton, 2005, Ndip et al., 2008; Mossad, 2008; Mungrue et al., 2009).

Recurrent URT1s in children constitute a serious problem world wide. Adults develop an average of two to four colds annually (Mossad, 2008). It has been reported that the majority of URT1s are of viral origin with rhinovirus, parainfluenza virus, coronavirus, adenovirus, respiratory syncytial virus and influenza virus accounting for most cases (Clark et al., 2004; Lykova et al., 2003). Apart from viruses, bacteria pathogens have been reported to cause URTI and these include *Haemophilus influenzae, Staphylococcus aureus, Streptococcus pneumoniae, Klebsiella pneumoniae, Pseudomonas aeruginosa* and some Enterobacteriaceae (Isenberg and D- Amato, 1985; Ndip et al., 2003).

The overuse and misuse of antibiotics for URTI in patients is widespread and fuelled by public attitudes and expectations heralding the emergence of resistance by microorganisms. It has been reported that susceptibility of pathogens to antibiotics varies with time and

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geographical location (EL-Sherkh, 1998; Ndip et al., 2002). The prescription of an antibiotic for URTI especially broad spectrum antimicrobial and second generation macrolides is a common practice in the medical profession and with the ever increasing tendency to buy antibiotic over- the- counter in the study area, the emergence of resistant strains of pathogens poses a great problem in the treatment and management of such pathogens (Ndip et al., 2001; Kollef et al., 2005; Hellinger, 2000; Stille et al., 2004; Nash et al., 2002)

Due to resistance to antibiotics by pathogens, recent research has been directed towards the use of traditional medicine/natural products for treatment and control of infections. Propolis is one of such products that is being tested on pathogens. It is a natural composite of balsam produced by honey bees (*Apis melifera*) from the gum of various plant. Bees collect vegetal exudate and form pellets with wax and products of their salivary gland. The resulting material is used to strengthen the nest, provide protection from microorganisms and as an embalming substance to cover the carcass of a hive invader (Gebara et al., 2002).

The medicinal and antimicrobial properties of propolis have been widely reported and have a long history (Dohrowoski et al., 1991; Forcht et al., 1993; Gebara et al., 1996; Park et al., 1998; Ikeno and Ikeno, 1991). Due to the increasing rate of antibiotic resistances by most bacteria of respiratory infections, treatment and management of URTIs which increases the risk of mortality and morbidity in patients have become difficult in the study area. Therefore, the antibacterial activity of propolis, a product from honey bee, which has been reported to act against *Escherichia coli, S. aureus, Candida albican* among others, is reported in this study for the possible use for the treatment and control of URTIs.

MATERIALS AND METHODS

Collection of sample

A total of 250 throat swabs samples from patients with upper respiratory tract infection attending the central hospital, Benin City, Nigeria were collected by trained personnel and samples were transported to the Microbiology Laboratory of the Department of Microbiology, University of Benin, Benin City, Nigeria for microbiological analysis. The diagnoses of URTIs for the purpose of this study are the common cold, acute, pharyngitis, acute tonsilitis sinusitis, acute otitis media and non- specific URTI. The patients age were between 15 - 30 years and they gave their informed consent for this study. The samples were collected between February 2008 - December 2008, a period that includes both the rainy and dry seasons in Nigeria. The ethical approval for the study was obtained from the Management of Edo State Ministry of Health, Nigeria.

Microbiological analysis

The method of Cheesbrough (2000) was used for the microbiological analysis. A loopful of each sample was inoculated into blood agar, chocolate agar and eosin-methylene blue (Biotec Lab, Ltd, UK) and incubated at 37°C for 24 - 48 h aerobically except for chocolate agar in which plates were incubated microaerophically. After incubation, macroscopic and microscopic examinations of colonies were carried out, sub-cultured on appropriate slants and stored at 37°C for biochemical and culture characterization for identification (Buchanan and Gibbon, 1974).

Extraction of propolis

Propolis was obtained from a honey bee market located in Ogharra, a community in Delta state close to Benin City, the study area. The market is known for the collection, processing and selling of honey bee products. The whole sample of propolis (30 g) was frozen, ground and homogenized prior to beginning extraction (Popova et al., 2005). The methods of Trusheva et al. (2007) and Silva et al. (2007) were used.

During extraction, propolis was ground to a fine powder and 2 g (dry weight) was mixed with 25 ml of 70% (v/v) ethanol and shaken in volumetric flask for 30 min. After extraction, the mixture was centrifuged and the supernatant was evaporated to produce the ethanolic extract of propolis (EEP) which was prepared at 1% with 70% (v/v) and the filterate diluted to 100 ml with 70% ethanol in a volumetric flask.

Antimicrobial activity of propolis

The Kirby-Bauer disc diffusion method was used as described (Bauer et al., 1966). Briefly, a small single well isolated colony was emulsified in 2 ml sterile saline in Bijou bottles and incubated at 37°C for 4 h to obtain the growing culture and the turbidity was adjusted to 0.5 Mc Farland standard. A sterile cotton swab with the adjusted suspension was used to evenly spread the entire surface of the Mueller- Hinton agar (Biotec Lab Ltd, UK) plates to obtain uniform inoculums. The plates were dried for 2 - 4 min.

Minimal inhibitory concentration (MIC) for propolis against the isolates were determined using ethanol extract of propolis (EEP) in serial concentrations: 0, negative, 0.25, 0.5, 1, 2, 4, 8 and 10 µg/ml. Control plates with serial concentration of ethanolic alcohol solution were also tested. All tests were performed in quadruplicate.

Propolis impregnated disc were applied to the surface of inoculated plates with sterile forceps, ensuring complete contact of disc with agar. The plates were incubated at 37°C for 16 - 18 h and examined for zones of complete inhibition to the nearest mm. Resistance and sensitivity to propolis was measured by the method of Baker and Breach (1980). When the antibiotic agent was 16 mm or higher, it was recorded as sensitive and resistant when less than 16 mm.

The Chi-square test was used to compare data. P values of < 0.05 were considered to be statistically significant.

RESULTS

Of the 250 throat swabs samples collected and examined 142 (56.8%) were males and 108 (43.2%) females. This was not significant ($p < 0.05$). The prevalence of bacterial isolated from throat swabs of patients is shown in Table 1. 64% (160) of the samples analyzed had positive cultures. The identified bacterial isolates included *H. influenzae, K. pneumoniae, S. pneumoniae, M. catarrhalis* and *S. pyogenes*. *H. influenzae* had the highest percentage prevalence of 20.8% followed by

Table 1. Prevalence of bacterial pathogens from throat swabs.

Species	Isolates number	% Prevalence
H. influenzae	52	20.8
K. pneumoniae	48	19.2
S. pneumoniae	30	12.0
M. catarrhalis	25	10.0
S. pyogenes	5	2.0
Total	160	64

Table 2. Prevalence of isolates by age (years)

Species	Age group (years)				
	15 - 18	19 - 22	23 - 26	27 - 30	Total
H. influenzae	30	19	2	1	52
K. pneumonia	28	6	4	0	48
S. pneumoniae	15	11	3	1	30
M. catarrhalis	15	10	0	0	25
S. pyogenes	3	2	0	0	5
Total	91	58	9	2	160
n =	120	78	40	18	

n = No. of patients in each age group.

Table 3. Minimum inhibitory concentration (MIC) of ethanolic extract of propolis (EEP).

Microorganisms	Zone of inhibition (mm)	MIC
H. influenzae	26	1.0
S. pneumoniae	32	2.0
M. catarrhalis	10	0.5
S. pyogenes	10	8.0

K. pneumoniae (19.2 %), *S. pneumoniae* (12.0%), *M. catarrhalis* (10 %) and *S. pyogenes* (2%). Of the 160 isolates, 68% were recovered from females while 32% from males.

Table 2 shows the prevalence of isolates in different age groups and the total number of isolates in each group. The highest rate of isolates was from the age group of 15 - 18 years (91) while the least was age 27 - 30 years (2). This was significantly higher than other age groups at $p > 0.05$. Also, all the isolates occurred more in age 15 - 18 years with *H. Influenzae* having a prevalence of 30 in 120 samples followed by *K. pneumoniae* (28) while *S. pyogenes* had a prevalence of 3 in this same age group. *M. catarrhalis* and *S. Pyogenes* were not isolated in age groups 23 - 26 and 27 - 30. *K. pneumoniae* was not isolated in age group 27 - 30 years.

The propolis extract showed antimicrobial activity against all 5 bacterial isolates (Table 3). All control plates including those with different ethanolic alcohol concentration and the negative controls, presented regular bacterial growth. Susceptibility was assessed with reference to CLSI guidelines (CSLI, 2005).

H. influenzae, K. pneumoniae, S. pneumoniae were more sensitive to propolis at an MIC of 1.0, 2.0 and 2.0 µg/ml respectively with zones of inhibition of 26, 32 and 30 mm each. *M. catarrhalis and S. pyogenes* were least sensitive with an MIC of 0.5 and 8.0 µg/ml, respectively and zones of inhibition of 10 mm each.

DISCUSSION

The study focused on the bacterial pathogens of URTIs in patients and their sensitivity to propolis. *H influenzae* was the most frequently isolated pathogen (20.8%). The isolation rates of 19.2, 12, 10, and 2% were also noted for *K. pneumoniae*, *S. pneumoniae*, *M. catarrhalis* and *S. pyogenes* respectively. Previous studies have reported these bacteria as significant cause of URTIs (Mungrue et al., 2009; Ndip et al., 2008; El-Sheikh, 1998). The higher rate of isolates from females (68%) than males (32%) may be attributed to their social and sex life. Females in

Benin City are known to be more promiscuous than their male counterparts due to their high needs for social materials.

Respiratory diseases have been reported to be more cause of death among children than diarrhoeae in developing countries, with *S. pneumoniae* among others being one of the main pathogenic micoorganism (Heruzo et al., 2002). *S. pneumonia* carriage has been reported to vary from 9 to 72% in different studies in sub-sahara Africa (Berkley et al., 2005; Hill et al., 2006; Mc Nally et al., 2006; Nyandiko et al., 2007). Although, we used the term 'pathogens' to refer to these bacteria, we recognize that these (and other) bacteria may be present in the nasopharynx without producing clinical disease. These bacteria are however potential pathogens.

Many cases of URT1s are known to respond to antibiotics. Severe pneumonia and meningitis have been reported to respond to chloramphenicol and benzylpenicillin, a mild pneumonia to trimethroprim- sulphamethoxazole and ampicillin (Scot et al., 2005). However, due to overuse and misuse of antibiotic for URTIs by patients, there is an increasing rate of antibiotic resistance by most bacterial pathogens. Bacterial resistance of between 20% to greater than 50% to amoxillin, cefuroxime, erythromymin by *S. pneumoniae* has been reported for all organisms associated with community acquired URTIs and increasing resistance of up to 30% to macrolides have also been reported (Pfaller et al., 2002; Hoban et al., 2003).

This increasing resistance has made it difficult for the treatment and management of URTI, which increases the risk for morbidity and mortality if treatment fails to eradicate the disease. Antimicrobial activity of propolis against URTIs has been reported (Focht et al., 1993; Mossad, 2008; Gabara, 2002). Popolis has been shown to have antimicrobial activity against bacterial pathogens of the oral cavity, respiratory tract and intestine tract and even against protozoa and viruses (Park et al., 1998; Steinberg et al., 1996).

The results of the antimicrobial activity of popolis showed that all the bacterial isolates were sensitive to EEP at different MIC concentrations. This may be due to the non-abuse of propolis by patients. Propolis is costly to buy and therefore is not within reach by low income earners and its antimicrobial activities have not been fully exploited and abused by both patients and healthy individual in the study area.

The major selective force favouring the emergency of antibiotics resistance is their extensive use ether due to their low cost or personal prescriptive. The result of this study is promising in the treatment and management of bacterial pathogens of URTLS. It has also been suggested and recommended that propolis be the antimicrobial agent of choice in the treatment of URTI, because of it antivirial activity which antibiotics lack (Gebara et al., 2002).

Our results shows that propolis extract presented "*in- vitro*" antimicrobial activity to *H. influenzae*, *K. pneumoniae*, *S. pneumoniae, M. catarrhalis* and *S. pyogenes*. Although the number of isolates used in this study may be too small to draw meaningful conclusion on susceptibility pattern, they however provide baseline data for future studies especially considering the fact that no such data have been reported in this environment. The result of these findings are therefore of clinical and epidemiological significance.

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