

Full Length Research Paper

Antibacterial activity of garlic and lime on isolates of extracted carious teeth

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Inhibitory activity of garlic (*Allium sativum* Linn.) and lime (*Citrus aurantifolia* Linn.) on seven bacterial species (*Streptococcus mutans*, *Lactobacillus acidophilus*, *Norcadia asteroides*, *Pseudomonas aeruginosa*, *Actinomyces viscosus*, *Staphylococcus aureus* and *Veillonella alcaligenes*) isolated from 240 extracted, carious teeth were investigated using standard techniques. The mean zones of inhibition for garlic, lime and a mixture of both, ranged from (15.0 ± 0.7) - (27.0 ± 1.0); (21.0 ± 1.0) - (27.0 ± 0.7) and (21.0 ± 0.7) - (30.0 ± 0.5) respectively, as compared with that for Gentamycin (18.0 ± 0.0) - (23.0 ± 0.5), indicating antibacterial activity. MICs for garlic, lime and a mixture of both, were 31.25 - 62.50 mg mL⁻¹; 1:2 v/v and 15.63 - 31.25 mg mL⁻¹ respectively, which coincided with the respective MBCs. The result of this investigation suggests that a paste made by blending garlic and lime could be used as a mouth wash in the treatment of dental caries, mouth-sore, sore-throat and also, be incorporated into toothpaste to prevent dental caries.

Key words: Garlic (*Allium sativum* Linn.), lime (*Citrus aurantifolia* Linn.), *Streptococcus mutans*, *Lactobacillus acidophilus*, *Norcadia asteroides*, *Pseudomonas aeruginosa*, *Actinomyces viscosus*, *Staphylococcus aureus*, *Veillonella alcaligenes*.

INTRODUCTION

Practitioners of traditional medicine believe that the constituents of plants are unique as they contain both active ingredients and "non-active" components that play a role in enhancing the well-being of their patients. A rekindled interest in the pharmaceutical importance of plants have led to the discovery and adoption of plant extracts which were commonly used in traditional medicine, as alternative source of remedy (Kabir et al., 2005). Tooth decay, a progressive destruction of enamel, dentine and cementum, mediated by microbial activity at the tooth surface (Steve, 1997), is not only excruciating but, economically debilitating. About 20 billion dollars is spent on restoring and replacing carious teeth in the United States alone, annually (Nester et al., 2004). Plant extracts formulations have therefore become a better alternative, especially in developing nations as treatments with these are relatively inexpensive.

Moreover, most antimicrobial agents that are currently in use have been rendered ineffective by the wide occurrence of multiple drug resistant strains of microbes that abound in the Nigeria environs (Owhe-Ureghe et al., 2000).

Lime (*Citrus aurantifolia* Linn.) is known to be an essential ingredient in the preparation of most herbal concoctions. It is used to suppress stomach ache, added to honey and palm oil to relieve cough, and the mesocarp is also used as a good facial scrub to prevent pimples (Oyagade et al., 1999). Its antimicrobial activities have also been described as various parts of the plant were found effective against gram-positive and gram-negative bacteria, as well as *Candida albicans* (Aibinu et al., 2007). The antimicrobial activity of the volatile oils of tangerine fruit peel (*Citrus reticulata*) have equally been described (Ayoola et al., 2008). Onyeagba et al. (2004), also studied the antimicrobial effect of garlic, ginger (*Zingiber officinale* Roscoe) and lime on *Staphylococcus aureus*, *Bacillus* sp., *Escherichia coli* and *Salmonella* sp., and confirmed the effectiveness of undiluted lime juice

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Table 1. Percentage prevalence of isolates in extracted carious teeth (N = 240).

Isolates	Number (% of occurrence)
<i>S. mutans</i>	204 (85.0)
<i>L. acidophilus</i>	96 (40.0)
<i>A. viscosus</i>	48 (20.0)
<i>P. aeruginosa</i>	36 (15.0)
<i>V. alcaligenes</i>	28 (11.7)
<i>N. asteroides</i>	24 (10.0)
<i>S. aureus</i>	24 (10.0)

on these organisms.

Garlic (*Allium sativum* Linn) on the other hand, has been reported to help prevent heart disease including atherosclerosis (Durak et al., 2002), as well as high blood pressure and cancer (Nishino, 1990; Imai, 1994). As early as 1858, Louis Pasteur observed garlic's anti-bacterial activity and it was used as an antiseptic to prevent Gas gangrene during World War II (Kock and Lawson, 1996). It has also been reasonably, successfully used in AIDS patients to treat *Cryptosporidium* infections in an uncontrolled study in China (Fareed et al., 2007).

The need to identify a common and cheap herbal remedy for the prevention and treatment of sore-throat, mouth sore and dental caries, especially in a developing nation, prompted us to investigate the therapeutic potentials of lime and garlic.

MATERIALS AND METHODS

Isolation and identification of isolates

Two hundred and forty (240) extracted, carious teeth were aseptically collected, each into a wide-mouthed screw-capped universal bottle containing 10 ml of sterile peptone water, from Tovo Dental clinic, Andrel Dental clinic both at Abraka, Nigeria and the Dentistry department of the Government's Central hospital Warri, Nigeria. Samples were immediately transported in ice-packed containers to the Microbiology laboratory of Delta State University, Abraka and incubated aerobically at 37°C for 24 h. Bacterial species were isolated from the peptone water broth, characterized and identified according to standard methods described in the Manual of Clinical Microbiology (Murray et al., 2007).

Preparation of extracts

Aqueous garlic extract was prepared according to methods previously reported by Onyeagba et al. (2004). 100 g of fresh, washed garlic cloves was macerated in a sterile, ceramic mortar. The homogenate was then filtered off with a sterile, muslin cloth and used directly for the sensitivity test.

Similarly, lime extract was prepared as previously described by Onyeagba et al. (2004) and Aibinu et al. (2007). A juice extractor was used to obtain the juice from 200 g fresh, clean lime fruits. This was subsequently filtered with a sterile, muslin cloth and used directly for sensitivity test. To ensure aseptic conditions, sterile gloves and face masks were worn and the entire extraction was carried out in a

media room of the Microbiology Laboratory, Delta State University, Abraka, Nigeria.

Standardization of isolates

A standard stock of the bacteria isolates were prepared by suspending a loop full of each microbial growth in about 10 ml of nutrient broth. After incubation at 37°C for 12 h, the turbidity was adjusted to be visually comparable with a 0.5 McFarland's standard giving a bacterial load of about $1 - 2 \times 10^8$ cfu mL⁻¹ (Murray et al., 2007).

Susceptibility test

The agar-well diffusion method prescribed by NCCLS (2000) was employed in the susceptibility testing. Suspensions of the bacterial isolates were made in sterile normal saline and adjusted to the 0.5 McFarland's standard. Each Mueller Hinton (MH) agar plate was uniformly seeded by means of sterile swab dipped in the suspension and streaked on the agar plate surface, and the plates left on the bench for excess fluid to be absorbed. Wells of 5 mm in diameter, 4 mm deep and about 2 cm apart were punched in the MH agar with a sterile cork-borer. Approximately 100 µl of the extracts were dropped into each well which filled them respectively to fullness. The setup were allowed to stabilize for 3 h before being incubated at 37°C for 24 h as described previously by Shahidi (2004) and Aibinu et al. (2007). The mean zones of inhibition were thereafter measured in mm, for all the individual isolates. A positive control well was equally filled with Gentamycin (32 µg mL⁻¹) while sterile, distilled water served as negative control.

Determination of MIC and MBC

The minimum inhibitory concentration (MIC) of the extracts was determined according to methods described by Shahidi (2004) and Kabir et al. (2005). Extracts were diluted to concentrations ranging from 7.82 to 500 mg mL⁻¹ (for garlic and a mixture of garlic with lime), and 1:16 to 1:1%v/v, for lime. To each dilution of garlic, lime and a mixture of both, in nutrient broth tubes were seeded 0.1 ml of the standard bacterial inoculum. Negative control tubes with no bacterial inoculation, were simultaneously maintained. Tubes were incubated aerobically at 37°C for 24 h. The lowest concentration of the extract that produced no visible bacterial growth (turbidity) was recorded as the MIC. Dilutions showing no visible growth for the MIC was sub-cultured onto a fresh MH Agar plate and incubated at 37°C for 24 h. The lowest concentration of the extracts yielding no growth on the MH plate was recorded as the minimal bactericidal concentration (MBC).

RESULTS AND DISCUSSION

Culture of the extracted, carious teeth investigated implicated 7 bacterial species to be associated with the various degrees of dental caries observed in this study. These include *Streptococcus mutans*, *Lactobacillus acidophilus*, *Norcadia asteroides*, *Pseudomonas aeruginosa*, *Actinomyces viscosus*, *S. aureus* and *Veilonella alcaligenes*. *S. mutans* (85.0%) and *L. acidophilus* (40.0%) were most prevalent as compared with other isolates (Table 1). This predominance is in agreement with findings of Hedge et al. (2005) where a prevalence of 87.4 and

Table 2. Mean \pm SD zones of inhibition of extracts on isolates.

Isolates	Gentamycin (32 $\mu\text{g mL}^{-1}$)	Water	Garlic (500 mg mL^{-1})	Lime (undiluted)	Garlic + Lime
<i>S. mutans</i> (n = 20)	20.0 \pm 0.0	-	20.0 \pm 0.5	23.0 \pm 1.0	23.0 \pm 1.0
<i>L. acidophilus</i> (n = 96)	18.0 \pm 0.7	-	20.0 \pm 0.7	21.0 \pm 1.0	21.0 \pm 0.7
<i>A. viscosus</i> (n = 48)	23.0 \pm 0.5	-	26.0 \pm 0.5	27.0 \pm 1.0	27.0 \pm 1.0
<i>P. aeruginosa</i> (n = 36)	21.0 \pm 0.7	-	19.0 \pm 1.0	22.0 \pm 0.7	23.0 \pm 1.0
<i>V. alcaligenes</i> (n = 28)	20.0 \pm 1.0	-	18.0 \pm 0.5	20.0 \pm 0.5	22.0 \pm 1.0
<i>N. asteroides</i> (n = 24)	18.0 \pm 0.0	-	15.0 \pm 0.7	21.0 \pm 1.0	23.0 \pm 0.5
<i>S. aureus</i> (n = 24)	22.0 \pm 0.0	-	27.0 \pm 1.0	27.0 \pm 0.7	30.0 \pm 0.5

- = No zone of inhibition.

Table 3. MICs of extracts on isolates.

Isolates	Garlic (mg mL^{-1})	Lime (% v/v)	Garlic + Lime (mg mL^{-1})
<i>S. mutans</i>	31.25	1:2	15.63
<i>L. acidophilus</i>	31.25	1:2	15.63
<i>A. viscosus</i>	31.25	1:2	15.63
<i>P. aeruginosa</i>	31.25	1:2	15.63
<i>V. alcaligenes</i>	62.50	1:2	31.25
<i>N. asteroides</i>	62.50	1:2	31.25
<i>S. aureus</i>	31.25	1:2	15.63

36.7% were recorded for *S. mutans* and *L. acidophilus*, respectively. *L. acidophilus* is the major culprit usually associated with carious teeth (Roger, 2008) and this is not unconnected with its elaborate acid production. Prevalence of *N. asteroides* and *S. aureus* were least, with 10.0% respectively. Since these bacteria convert sugars into acid such as lactic acid through the glycolytic process of fermentation (Holloway, 1983), their role in demineralization and ultimately, formation of cavities, is not in doubt.

Previous authors have described the prevalence of *S. mutans* and *Lactobacillus* in carious teeth (Nishikawara et al., 2006). However, Aas et al. (2008) used polymerase chain reaction (PCR) amplified 16S rRNA genes to identify bacteria isolates from the plaque of carious teeth. They concluded that bacterial species other than *S. mutans*, e.g., species of the genera *Veillonella*, *Lactobacillus*, *Bifidobacterium* and *Propionibacterium*, low pH non-*S. mutans* streptococci, *Actinomyces* sp., and *Atopobium* sp., likely play important roles in caries progression.

The diameter of the zone of inhibition ranged from 15.0 \pm 0.7 to 27.0 \pm 1.0 mm for garlic and 20.0 \pm 0.5 to 27.0 \pm 0.7 mm for lime, as compared with 18.0 \pm 0.0 to 22.0 \pm 0.0 mm for gentamycin (Table 2), at the various concentrations used. Results indicate a considerable antibacterial activity of garlic and lime. The combined extracts were most effective against *S. aureus* 30.0 \pm 1.5 mm but least effective against *L. acidophilus* 21.0 \pm 0.7 mm (Table 2). *L. acidophilus* is the major culprit

implicated with dental caries (Hardie, 1982; Roger, 2008) with susceptibility equally comparable with that of gentamycin with ZI = 18.0 \pm 0.0 mm. Previous researchers have described the antibacterial activity of garlic against multi drug resistant (MDR) *S. mutans* (Fani et al., 2007), as well as methicillin resistant *S. aureus* and MDR *P. aeruginosa* (Tao and Yin, 2001).

The MIC of garlic against the test isolates ranged from 31.25 - 62.5 mg mL^{-1} (Table 3). This is slightly higher compared with the MIC of garlic extract against *S. mutans* which ranged from 4 - 32 mg mL^{-1} as previously reported by Fani et al. (2007). This difference in value could be largely attributed to the extraction process. Generally, a synergistic effect was observed for a combination of lime and garlic that shows MIC decrease to a range of 15.63 - 31.25 mg mL^{-1} (Table 3).

Considering *in vitro* data obtained in this study, there is a synergistic effect of antimicrobial activity from the combination of garlic and lime, against isolates from carious teeth. The result of this investigation suggests that a paste made by blending garlic and lime could be used as a mouth wash in the treatment of dental caries, mouth sore, sore throat and also, be incorporated into toothpaste to prevent dental caries. Further studies on toxicity tests are recommended.

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