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The inhibitory effect of different chemical food preservatives on the growth of selected food borne pathogenic bacteria

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The inhibitory effect of different chemical food preservatives (potassium metabisulphite, sodium benzoate, citric acid, ascorbic acid and potassium sorbate) on the growth of selected food borne pathogenic bacteria (*Staphylococcus aureus, Klebsiella aerogenes, Proteus mirabilis, Pseudomonas aeruginosa and Escherichia coli*) were investigated using agar diffusion technique. The concentrations used on test bacteria ranged from 0.125 - 1.5 mg/ml. The minimum inhibitory concentrations (MIC) of the five preservatives were 0.5, 1.5, 1.5, 1.5 and 1.5 mg/ml for potassium metabisulphite, sodium benzoate, citric acid, ascorbic acid and potassium sorbate, respectively. The zone of inhibition of the test bacteria ranged from 4.0 - 21 mm. *S. aureus* was susceptible to all the five chemical food preservatives. All the MIC of chemical food preservatives used against selected pathogenic bacteria was below the acceptable daily intake (ADI) mg/kg body weight/day. The MIC of citric acid ascorbic acid has no ADI limit in the body.

Key words: Chemical preservatives, pathogenic bacteria, minimum inhibitory concentration, acceptable daily intake (ADI), inhibition zone.

INTRODUCTION

Food is defined as any chemical substance which when eaten, digested and absorbed by the body, produces energy, promotes growth and repairs the body tissues and regulates these processes (Olunlade et al., 2010). Foods are not only of nutritional value to those who consume them but often are ideal culture media for microbial growth. Chemical reactions that cause offensive and sensory changes in foods are mediated by bacteria that use food as a carbon and energy source. Some of the major bacterial genera which cause food borne infection and intoxication include *Staphylococcus aureus, Klebsiella aerogenes, Proteus mirabilis, Pseudomonas aeruginosus and Escherichia coli* e. t. c. (Pundir and Jain, 2011). Food, despite it supplies nutrients to the body; it can also be responsible for ill health (Adams and Moss, 1999). The ill health occurs as a result of ingestion of

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food borne pathogens (Frazier and Westhoff, 2002). The ingestion of food borne pathogen leads to food borne diseases which are food intoxications and infections (Frazier and Westhoff, 2002). The following micro-organisms (*S. aureus, K. aerogenes, P. mirabilis, P. aeruginosus and E. coli* etc) are normally implicated in food borne diseases (Adeleke and Oladapo, 2010). Based on the type of microorganisms responsible for food borne diseases, these diseases therefore, can be classified as being of bacterial, viral, fungal or protozoal. These diseases cause serious problems on the health of man. There is therefore, the need to control the growth of pathogenic microorganisms in foods. One way by which this is achieved is through the use of chemical preservatives. These chemicals are substances of no nutritional significance (Joshua, 2000). They are added to foods as antimicrobial agents to preserve them from deterioration and extend their shelf life (Jay, 2005). These chemicals should not have toxic effect on human cells (Mahindru, 2000). It has to be economical and should not have an effect on the taste and aroma of the original food, or any substance in food, nor encourage the development were of resistance strains are killed rather than inhibit micro-organisms. Most preservatives are inhibitory at acceptable level (Frazier and Weshtoff, 2002). Chemicals that have been used in the preservation of foods are including sodium chloride, sodium nitrate and nitrite, sodium benzoate, ascorbic acid and propionic acid (Mahindru, 2000). These chemical agents are employed to prevent microbial growth in food (Prescott et al., 2002). Some acids and salts especially benzoic and ascorbic acid and its salts are effective inhibitors of microbial growth and are intentionally added to many foods as preservatives (Dziezak, 1986). Other acids including acetic acid, fumaric acid, proponic acid and lactic acid are added to foods to prevent or delay the growth of pathogenic bacteria (Dziezak, 1986; Greer and Dilts, 1995; Podolak et al., 1996).

This study investigated the inhibitory effect of some chemical preservatives on the growth of selected food-borne bacterial pathogens.

MATERIALS AND METHODS

Collection of chemical preservatives and clinical isolates

Five chemical preservatives were used for the experiments. These were sodium metabisulphite, sodium benzoate, citric acid, ascorbic acid, and potassium sorbate. They were collected from Food Technology Department, Osun State Polytechnic, Iree, Osun State while the clinical isolates of *S. aureus, K. aerogenes, P. mirabilis, P. aeruginosa and E. coli* were collected from Ladoke Akintola University Teaching Hospital Complex, Oshogbo, Nigeria.

Confirmation of the identity of collected organisms

The method described by Cheesbrough (2002) was adopted to confirm the identity of the microorganisms adequately. The isolates were cultured on various selective and differential media to confirm their identity on Grams staining, motility and biochemical tests.

Preparation of different concentrations of chemical preservatives

Five different concentrations ranging from 0.0125 to 1.5 mg/ml of each chemical preservative were weighed and poured into the McCartney bottles containing 10 ml of sterile distilled water. Each concentration prepared was labeled and shaken vigorously (Christian, 1994; Doughari, 2006).

Antibacterial screening of the chemical preservatives

The agar plate diffusion method of Nostro et al. (2000), Xavier et al. (2003), Ajayi (2005), Omoya and Akharaiyi (2010) were used. The medium used was nutrient agar, which supports the growth of bacteria. Then with the aid of sterile needle and syringe pipette, 1.0 ml of each broth culture of standardized bacteria of 10^6 was added into sterilized plate and about 20 ml sterile nutrient agar, which had already cooled to 45°C ± 2, was poured aseptically, mixed and allowed to solidify. With the aid of sterile 6 mm cork borer, well were bored on the agar surface to the edge of the plate and labelled accordingly. Each well was then filled up with the chemical preservative concentration as prepared. The prepared plate cultures were incubated at 37°C ± 2 for 24 h. Clear zones of inhibition around the well indicated the sensitivity of the test bacteria to each of the chemical preservatives and diameter of the clear zones of inhibition was taken as an index of the degree of sensitivity by measuring with caliper. The venial caliper was placed on the transparent meter rule for proper accuracy of inhibition zone measurement (Pundir and Jain, 2011).

RESULTS AND DISCUSSION

Food preservation implies putting microorganisms in a hostile environment in order to cause their death. In this study, different concentrations of different chemical food preservatives were used to inactivate selected food borne pathogenic bacteria by agar diffusion technique. Figures 1 to 5 show the diameter zone of inhibition mediated by different concentrations ranging from 0.0125 to 1.5 mg/ml of food chemical preservatives (potassium metabisulphite, sodium benzoate, citric acid ,ascorbic acid and potasium sorbate) on the five test pathogenic bacteria (*S. aureus, K. aerogenes P. mirabilis, P. aeroginosa* and *E. coli*). The zones of inhibition were observed around the wells, this indicated antibacterial activities of the chemical preservatives. Different organisms have demonstrated different rankings for the inhibiting effects of chemical preservatives (Matsuda et al., 1994). The sensitivity of the different test organisms to different concentrations of chemical food preservatives was shown by zones of inhibition after 24 h of incubation; this is depicted in Figures 1 to 5. The absence of zones of inhibition around each well signified resistance. It was observed that the water used in preparation of these chemical preservatives was used as control which did not inhibit the growth of any of the test bacteria. *The S. aureus* had highest zone of inhibition of 21 mm for potassium sorbate at the

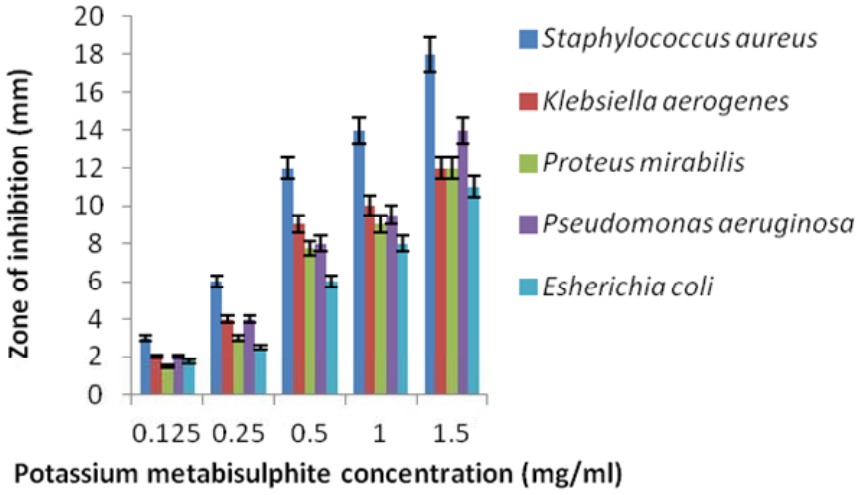

Figure 1. Diameter zone of inhibition (mm) mediated by different concentration (mg/ml) of potassium metabisulphite on selected food borne pathogenic bacteria.

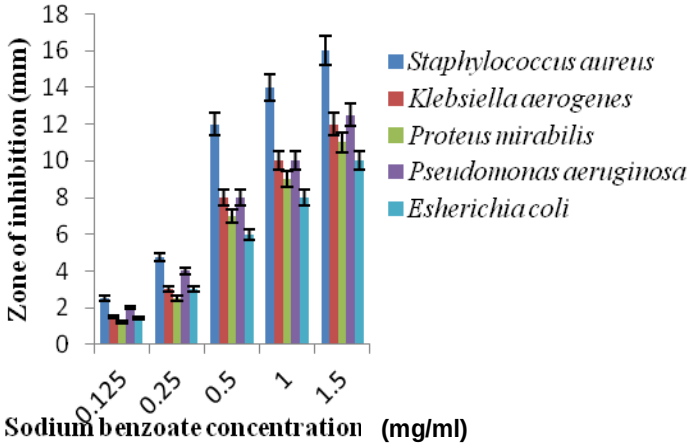

Figure 2. Diameter zone of inhibition (mm) mediated by different concentration (mg/ml) of sodium benzote on selected food borne pathogenic bacteria.

concentration of 1.5 mg/ml. This is an indication that *S. aureus* is sensitive to the chemical preservative as reported by Nanda (2005) that any zone of inhibition lesser than 4.0 mm is resistant, 4.0-12.0 mm are intermediate while any zone of inhibition that is more than 12.0 mm is sensitive to chemical food preservatives. The effect of potassium sorbate at various concentrations namely: 0.0125 to 1.5 mg/ml was tested for the inhibition of growth of the bacterial *S. aureus, K. aerogenes P. mirabilis, P. aeroginosa* and *E. coli* assay method and the results were summarized in Figures 1 to 5. The inhibition zone area increased with increase in the concentration of potassium sorbate for all the five bacterial cultures. Among the five bacterial cultures tested, potassium sorbate highly inhibited the growth of *S. aureus* by showing 21 mm zone of inhibition followed by *P. mirabilis*, (15 mm). The least zone of inhibition was seen in *E. coli* (12 mm) and *P. aerogenosa* (13 mm) and this is in line with the work of Jageethadevi et al. (2012) that reported inhibition zone increased with increase in concentration of chemical food preservatives. Then, higher concentration of chemical preservatives had greater inhibitory power on the microbial growth (Oyawoye et al., 1999). The zone of inhibition depends on Gram reaction of the preservatives. Gram positives bacteria are more sensitive than Gram negative to the chemical preservatives (Adams and Moss, 1999). *S. aureus* is a Gram positive bacteria while the rest test bacteria (*K. aerogenes P. mirabilis, P. aeroginosa* and *E. coli*) are Gram negative. The cell wall of Gram positive bacteria compose of peptidoglycan which is an essential polymer and interference with its synthesis or structure leads to loss of cell

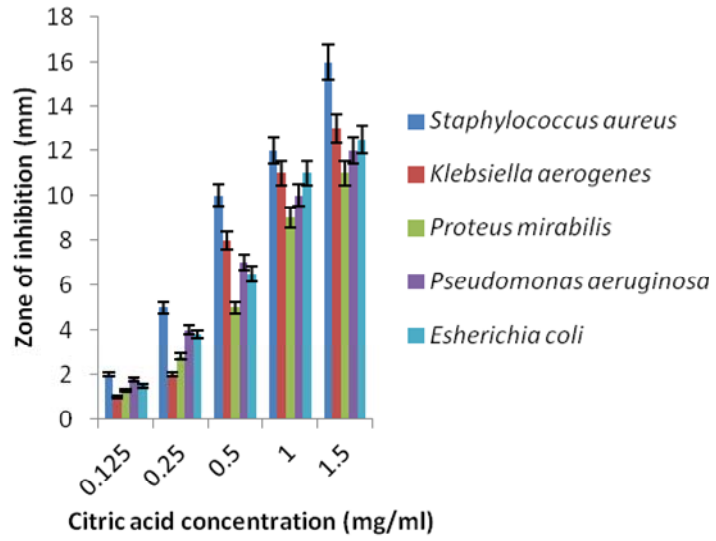

Figure 3. Diameter zone of inhibition (mm) mediated by different concentration (mg/ml) of citric acid on selected food borne pathogenic bacteria.

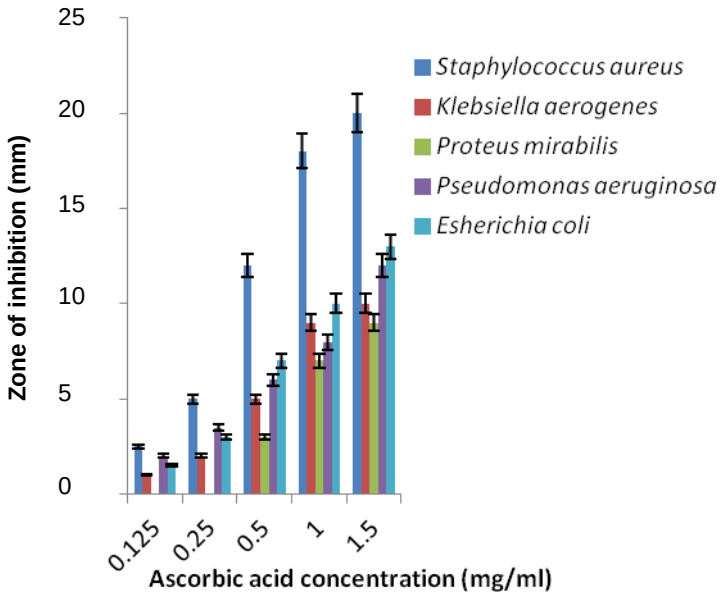

Figure 4. Diameter zone of inhibition (mm) mediated by different concentration (mg/ml) of ascorbic acid on the growth of selected food borne pathogenic bacteria.

shapes and integrity followed by bacterial death (Willey et al., 2011). The Gram negative bacteria are known to possess lipopolysaccharide on the outer membrane. In terms of susceptibility to chemical preservatives, they differ widely between the microorganisms (Mailard, 2002). The complex nature of the outer membrane of Gram negative bacteria has been reported to act as permeability barriers (Nikkado and Vara, 1985; Appleton and Lange, 1994; Willey et al., 2011). The citric acid and ascorbic acid concentration has no limit for the acceptable daily intake in the body. While the prominent zone of inhibition mediated by these chemical preservatives (potassium metabisulphite, sodium benzoate and potassium sorbate) concentrations were below the acceptable daily intake in the body (Mahindru, 2000).

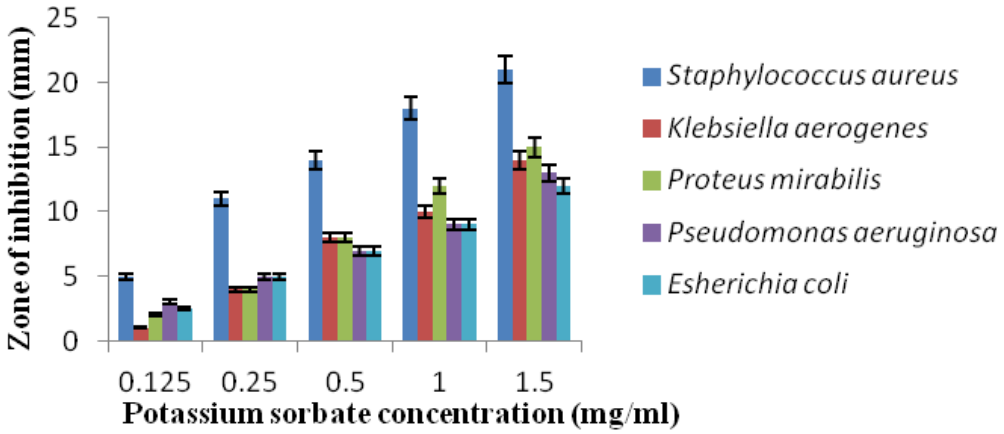

Figure 5. Diameter zone of inhibition (mm) mediated by different concentration (mg/ml) of potassium sorbate on the growth of food borne pathogenic bacteria.

Conclusion

Food preservation implies putting microorganisms in a hostile environment in order to inhibit their growth or shorten their survival or cause their death. The chemical preservatives (potassium metabisulphite, sodium benzoate, citric acid ,ascorbic acid and potasium sorbate) are effective in the control of selected pathogenic bacteria (*S. aureus, K. aerogenes P. mirabilis, P. aeroginosa* and *E. coli*) causing food poisoning and infection. *S. aureus* is highly susceptible to all chemical preservatives used in this study. The minimum inhibitory concentrations (MIC) of the five preservatives were 0.5, 1.5, 1.5, 1.5 and 1.5 mg/ml for potassium metabisulphite, sodium benzoate, citric acid, ascorbic acid and potassium sorbate, respectively.

It can be recommended that the above concentrations of the chemical preservatives could be used to preserve against food borne pathogens. In many cases, a concentration sufficient to result in the lyses of bacterial food poisoning growth may be all that is required to achieve a safe food product. Therefore, employing these chemical preservatives against some food poisoning bacteria provides an exciting potential for the future.

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