Antimicrobial activity of aqueous and ethanol extracts of nine Nigerian spices against four food borne bacteria.

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Abstract: The antimicrobial activity of the aqueous and ethanol extracts of nine Nigerian spices (Xylopia aethiopica, Myristica fragrans, Aframomum sceptum, Garcinia cola, Zingiber official, Piper guanine, Allium cepa, Vanilla fragrans and Opium gratissimum) against Staphylococcus aureus, Klebsiella pneumoniae, Proteus vulgaris and Streptococcus faecalis was evaluated by determining the minimum inhibitory concentrations (MIC) of the extracts. By and large, all the extracts possessed antimicrobial properties with the MIC of the extracts in the range of 5 mg/ml to 22 mg/ml. The ethanol extract of V. fragrans (MIC=5 mg/ml – 10 mg/ml) was the most potent of all extracts while the least potent of the extract was M. fragrans (MIC=18 mg/ml – 22 mg/ml).

Key word: Antimicrobial, extracts, aqueous, ethanol, spices, bacteria, minimum inhibitory concentrations.

Introduction

There has been an increasing consumer demand for foods free or with low, if any, added synthetic preservatives because synthetic preservatives could be toxic to humans (Bedin et al., 1999). Concomitantly, consumers have also demanded for wholesome and safe food with long shelf lives. These requirements are often contradictory and have put pressure on the food industry for progressive removal of chemical preservatives and adoption of natural alternatives to obtain its goals concerning safe food with long shelf lives. This resulted in the use of new technologies, which include modified atmosphere packaging, natural antimicrobial compounds etc., for food preservation (Brull and Coote, 1999).

Numerous naturally occurring antimicrobials are present in animal and plant tissues (Smid and Gorris, 1999), where they probably evolved as part of the defence mechanisms of the host against microbial invasion. There are many plants that demonstrate antimicrobial activity (Beales, 2002) and these plants have found application in the food industry as antibacterial and antifungal agents (Lanciotti et al., 2004). Being plants, spices appeal to consumers who question the safety of synthetic preservatives (Sagdic, 2003). Although, the primary purpose of spices is to impart flavour and piquancy to food, the medicinal, antimicrobial and antioxidant properties of spices have also been exploited (Souza et al., 2005). The antimicrobial activity of spices is documented and interest continues to the present (Nychas, 1995; CAST, 1998; Cosentino et al., 1999; Domans and Deans, 2000; Ristori et al., 2002; Beales, 2002; Radhakrishnan-Sridhar and Velusamy-Rajaopal, 2003).
Literature search reveals that little information is available emphasizing the antimicrobial activity of Nigerian spices against food borne disease-causing microorganisms. As a result of this knowledge gap, this present study represent an attempt aimed at investigating the antimicrobial activity of nine Nigerian spices (*Xylopia aethiopica*, *Myristica fragrans*, *Aframomum cryptum*, *Garcinia colacca*, *Zingiber officinalis*, *Piper guineense*, *Allium cepa*, *Vanilla fragrans* and *Ocimum gratissimum*) against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Streptococcus faecalis*. These four microorganisms are important food borne pathogens, which have been implicated in a number of health disorder (Uraih, 2004). Thus, this study will provide information on the possible application of these spices as antimicrobial agents in food industry.

**Materials and Methods**

Preparation spice extract

The spices were purchased from Oba Market in Benin City, Nigeria in the month of November, 2007. The genus and species of spices were confirmed by comparison with herbarium reference materials housed in the Department of Botany, University of Benin, Benin City, Nigeria. Spices were inspected for foreign materials and any visible dirt and damaged parts were removed. They were sun dried to a constant weight and milled to a fined powder with the aid of a stainless steel mill. The powdered spice (100 g) was soaked in 400 ml of distilled deionizer water to prepare the aqueous extract and in 400 ml of absolute ethanol to prepare the ethanol extract. The suspension was stirred at 150 rpm at room temperature for 24 h after which it was filtered with the aid of a Whatman No 1 filter paper. The residue was re-extracted with 400 ml of the solvent as described. The combined extract were then rotary evaporated to dryness at 40 °C, redissolved in the corresponding solvent to obtained extracts of several concentrations (5 mg/ml, 10 mg/ml, 20 mg/ml, 30 mg/ml and 40 mg/ml) and stored at 4 °C prior to use.

Preparation of innocula

The microorganisms (*Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Streptococcus faecalis*) were obtained the stock culture collection of the Microbiology Department of the University of Benin Teaching Hospital (UBTH), Benin City, Nigeria. Each bacterial isolate was tested for viability by resuscitating the organism in a buffered peptone broth, after which it was subcultured into nutrient agar medium and incubated at 37 °C for 24 h.

Screening of antimicrobial activity of spice extract

The antimicrobial activity of the spice extracts against the selected microorganisms was evaluated by the cup-plate agar diffusion method (Ebi and Ofoefule, 1997; Ijeh et al., 2005). A 20 ml of the molten nutrient agar was seeded with 0.2 ml of broth cultures of the test organisms in sterile Petri dishes. The Petri dishes were rotated slowly to ensure a uniform distribution of microorganisms. The nutrient agar was left to solidify in the dish. With the aid of a sterile cork borer, cups of 8.0 mm diameter were made in the nutrient agar. The extracts were inoculated into the cups with the aid of a sterile Pasteur pipette. The dishes were allowed to stand for 30 min at room temperature to allow for proper diffusion of the extract to take place. The plate was then incubated at 37 °C for 24 h. At the end of the incubation period, inhibition zones formed on the medium were measured in mm. The minimum inhibitory concentration (MIC) in mg/ml was determined by comparing the different concentrations of a particular extract that have different zones of inhibition and then selecting the lowest concentration for each extract (Ijeh et al. 2005).

**Results and discussion**

The antimicrobial activity of the aqueous and ethanol extract of nine spices was tested against two species of Gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus faecalis*) and two species of Gram-negative bacteria (*Klebsiella pneumoniae* and *Proteus vulgaris*). These bacteria present various undesirable attributes of virulence that make them some of the most serious threats for food safety (Proctor and Davis, 2000). As can be seen from the results in Figure 1 and 2, all the aqueous extracts exhibited lower MIC than their corresponding ethanol extracts; the exception was the aqueous extract of *V. Fragrans*, which had a higher MIC than its ethanol fraction. The implication of this present finding is that the aqueous extract of these spices had more potent activity against the test bacteria than the
corresponding ethanol extract and so have more potential as an antimicrobial agent than their ethanol fraction. In their study, Ijeh et al. (2005) reported that aqueous extract of *X. aethiopica* was a better antimicrobial agent against *S. aureus* than the ethanol fraction. Our results, which agreed with the report of Ijeh and his co-workers, also justified the folkloric use of some of these spices, such as *X. aethiopica*, in the treatment of dysentery and diarrhea. This finding also suggests that the antimicrobial compounds in *V. fragrans* might be water insoluble while the antimicrobial compounds in the other spices might be water soluble.

The results also showed that the Gram-negative bacteria were relatively more resistant to the test extracts than the Gram-positive bacteria with the exception of *S. aureus*, which was the least sensitive to the aqueous extract of *V. fragrans* (MIC=20 mg/ml). Generally, Gram-negative bacteria have been reported to be more resistant than Gram-positive bacteria to the essential oils, which are antimicrobial agents (Chanegriha et al., 1994).

The maximum antibacterial activity (MIC=5 mg/ml) was shown by the ethanol extract of *V. fragrans* against *K. pneumoniae*. Furthermore, the ethanol extract of *V. fragrans* also had the maximum antibacterial activity against *P. vulgaris* (MIC=10 mg/ml), *S. aureus* (MIC=6 mg/ml) and exhibited one of the most potent activity against *S. faecalis* (MIC=8 mg/ml). As can be seen from the figures, the ethanol extract of *V. fragrans* exhibited the most potent antibacterial activity among the tested extracts.

This suggests the possible potential use of the ethanol extract of *V. fragrans* as an antimicrobial agent and as a preservative in the food industry. The minimum potent activity (MIC=22 mg/ml) was shown by the ethanol extract of *M. fragrans* against *K. pneumoniae*; in fact, ethanol extract of *M. fragrans* had the least antimicrobial activity of all the extracts.

All the tested aqueous extracts except that of *V. fragrans* remarkably inhibited the growth of *S. aureus* (10 mg/ml ≤ MIC ≥13 mg/ml). The results also showed that *P. vulgaris* had low sensitivity (MIC=20 mg/ml) to ethanol extract of *M. fragrans* and *A. sceptum*. This low sensitivity (MIC=20 mg/ml) was also shown by *K. pneumoniae* to the aqueous fraction of *A. sceptum*; and *S. aureus* to the aqueous extract of *V. fragrans*.

Previous researchers have shown the bacteriostatic and bactericidal effects of *A. cepa* and *Z. officinale* (Leuchner and Zamparini, 2002 and Sakagami et al., 2000). Moreover, Ijeh et al., have also shown the potency of ethanol and aqueous extracts of *X. aethiopica* against some common food borne pathogens such as *E. coli* and *S. aureus*. The results of this study agreed with the findings of previous workers. By and large, all the tested extracts exhibited antimicrobial activity against these bacteria and so could be used as microbial growth inhibitors in the foods.

**References**


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