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Bacteriological Assessment of *Musa paradisiaca* and *Cymbopogon citratus* leaf Extracts *on Staphylococcus aureus* Isolated from Seasoned Yaji sold in Avyi Ward, Wukari

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ABSTRACT

The antimicrobial properties of plants have been well documented globally. Scarcely any assessed their activities against potentially food poisoning organisms directly isolated from the contaminated foods. This present study was designed to evaluate the antimicrobial effects of extracts of Banana (*Musa paradisiaca*) and Lemon grass (*Cymbopogon citratus*) leaves on *Staphylococcus aureus* isolated from *Yaji,* a Nigerian seasoned spice. The standard organism used was *S. aureus* ATCC 6538. The cold and hot water extracts of the fresh and dried leaves of both plants showed no effectiveness against the isolates. The ethanolic extracts of fresh Lemon grass leaves showed no effectiveness against *S. aureus*. However, fresh Banana leaf extracts at 100mg/ml produced inhibition zones of 22mm and 26 mm against *S. aureus* ATCC 6538 and the test *S. aureus* respectively. The dried Lemon grass leaf extract at 100mg/ml, produced inhibition zone diameters of 13mm and 14mm against the typed *S. aureus* strain and the test *S. aureus* respectively while at 50mg/ml, it produced an inhibition zone of 11mg/ml against *S. aureus* ATCC 6538 only. This study established the efficacy of fresh and dried ethanolic leaf extracts of Banana and Lemon grass respectively against *S. aureus.* The extracts of these plants should be further analyzed to isolate the specific phytochemicals responsible for their antibacterial properties. This research and eventual commercialization of the bioactive agents will be an added arsenal in the treatment of staphylococcal food infection and poisoning.

INTRODUCTION

Food borne infection have been reported with significant morbidity worldwide and still pose a risk to humans (Uwaezuoke

et al., 2007; Kadariya *et al.*, 2014; Abdulmutalib *et al.*, 2015; Addis and Sisay, 2015). In Nigeria, the contamination of food has been locally associated with evil spirit, malice or curses (Osagbemi, *et al.*, 2010) even though it was most probably due to the unhygienic practices during production and commercialization. The main sources of bulk milk contamination are milking equipment, environment and food handlers carrying enterotoxin producing *S. aureus* in their noses, respiratory secretions or on their hands (Argudin *et al.*, 2010; Okolie *et al.*, 2012; Omemu *et al.*, 2014; Awujo *et al.*, 2022),

Staphylococcal food poisoning is common in humans and animals as a result of the ingestion of enterotoxins in food performed by enterotoxigenic strains of Gram- and coagulase positive *Staphylococcus aureus* (Argaw and Addis, 2015). Poultry, meat, dairy and their products, have been frequently incriminated in staphylococcal intoxication (Dzirba and Osek, 2011). Plants are good sources of antimicrobial agents. The use of plant extracts with known and unknown antimicrobial effects on clinical isolates has gained attention due to their availability, cost, biodegradability, effectiveness, specificity and low toxicity (Awujo *et al.*, 2007; Okoli *et al.*, 2007; Das *et al.*, 2010). The monoecious herb, Banana, *Musa paradisiaca*, a hybrid between *Musa acuminata* and *Musa balbisiana*, belongs to the *Musaceae* family and is one of the most popular fruits distributed globally. Traditionally, it is used for dressing of wounds, ulcers and for treating anaemia, diarrhoea, dysentery and intestinal colitis among other disorders. Its neurogenic, antioxidant and antimicrobial activities have also been reported (Okoli *et al.*, 2007; Ekpenyong *et al.*, 2014).

Lemon grass, *Cymbopogon citratus*, belongs to the *Graminae (Poaceae)* family and is a perennial plant that is widely distributed

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worldwide (Francisco *et al.*, 2011). Traditionally, tea made from lemon grass leaves is popular in South Africa, Asia and West Africa for its flavour. When squeezed, the leaves' aqueous extracts produce a yellow or amber coloured aromatic, essential oil that is variously utilized as carminatives, antiseptics, antipyretics, anti-dyspeptics, anti-inflammatory and antimicrobial substances (Srivastava *et al.*, 2013).

Interests in new plant derived antimicrobials have soared due to the need to overcome emerging antibiotic resistance and rising cost of antibiotics. However, their quantity and quality may directly or indirectly be influenced by the bioactive constituents, botanical source, time of harvesting, stage of development, species differences, method of extraction or preparation of the infusion and decoction, technique used in drying the leaves, agro-climatic factors such as soil salinity and water content (Ekpenyong *et al.*, 2014). The extraction and phytochemical screening of bioactive agents from plants permits the demonstration of their physiologic activities. In this regard, this present study was carried out to test the susceptibilities of two different *Staphylococcus aureus* isolates to aqueous and ethanolic extracts of *M. paradisiaca* and *C. citratus* leaves.

MATERIALS AND METHODS

Study Area

Wukari is one of the fifteen wards of Wukari Local Government Area (LGA) of Taraba State, Nigeria. It is largely a sub urban and multi-religious town dominated by the Jukun tribe. It lies on Longitude $7^0$57' and Latitude $9^0$42' East of the equator, has a land area of 4,308km^2 and a population of 241,546 people. Most of the residents are farmers while others rear cattle, fish and trade (Oruonye and Abbas, 2011).

Plants

The banana (*M. paradisiaca*) and Lemon grass (*C. citratus*) leaves were botanically identified in the Department of Biology, Federal University Wukari, Taraba State, Nigeria, before collection and laboratory extraction.

Standard and Test Organisms

A typed *Staphylococcus aureus* ATCC 6538 strain was collected from the National Veterinary Research Institute (NVRI) Vom, as pure culture isolates on agar slant while the test *S. aureus* was isolated from locally made seasoned Yaji by culturing directly on mannitol salt agar plates using the quadrant streak plate technique. Pure isolates were characterized using conventional biochemical tests to ensure that no variation with the typed organism existed before experimentation (Fagbemi *et al.*, 2009; Abah and Egwari, 2011).

Yaji

Locally prepared Yaji was obtained from a market vendor at Avyi Ward in Wukari, Taraba State. It consisted of a mixture of spices and additives including Ginger (*Zingiber officinale*), Garlic (*Allium sativum*), Cloves (*Syzigium aromaticum*), Alligator pepper (*Aframomum melegueta*), Red pepper (*Copsicum annuum*), African black pepper (*Piper guineense*), African Negro pepper, Table salt and "Maggi" seasoning.

Extraction Techniques

Extracts were obtained using cold and Soxhlet extraction methods described by Awujo *et al.* (2007), Fagbemi *et al.* (2009) and Abah and Egwari (2011). Equal portions of fresh plant leaves were cleaned, chopped into a constant weight and crushed in a sterile mortar to increase their surface areas. Cold preparations were made with 100g of each fresh leaves by completely

immersing each of them in 100ml of sterile distilled water for 24 hours. The mixtures were separately filtered using the Whatman No. 1 filter paper and the water separated from the extracts using a rotary evaporator before use (Awujo *et al.*, 2007). An equal portion of each fresh leaves was allowed to air-dry after which they were crushed into 100g of their powders. Hot water and ethanolic extracts were obtained from each of them using a conventional Soxhlet system extraction technique (Fagbemi *et al.*, 2009; Abah and Egwari, 2011). The extracts were equally but separately weighed into 100mg, 50mg and 25mg and individually dissolved in 1ml of DMSO to respectively yield concentrations of 100mg/ml, 50mg/ml and 25mg/ml.

Standardization of Inoculum for Antimicrobial Assay

Test isolates were sub-cultured on nutrient agar plates, incubated aerobically for 24 hours and diluted in normal saline to a density of 0.5 McFarland Standard (Akinsami *et al.*, 2015).

Antimicrobial Susceptibility Testing

This was carried out using the Well-in-Agar diffusion method (Awujo *et al.*, 2007; Lehtopolku *et al.*, 2012; Ewansiha; *et al.*, 2012). To ensure that the isolates were in their exponential phase of growth, they were sub-cultured aseptically into nutrient broth and incubated for 6-8 hours. Subsequently, they were inoculated on freshly prepared Mueller-Hinton agar after which different concentrations (100mg/ml, 50mg/ml and 25mg/ml) of the extracts were introduced into the agar wells. Pre-diffusion of the extracts was allowed for 30 minutes before incubation at a growth temperature for 18-24 hours. Thereafter, standard method was used to determine their minimum inhibitory concentrations (MIC) while the diameters of clear zones of inhibition were measured with a measuring ruler from the edge of

the well to the end of the clear zone of inhibition. Measurements were not taken when no clear zone of inhibition were observed.

RESULTS

The hot and cold water extracts of the fresh and dried leaves of each plant showed no activity against the two isolates (Tables 1 and 2). The ethanolic extracts of fresh Lemon grass leaves showed no effectiveness against *S. aureus*. However, ethanolic extracts of fresh Banana leaf extracts at 100mg/ml produced inhibition zones of 22mm and 26 mm against *S. aureus* ATCC 6538 and the test *S. aureus* respectively (Table 1). Dried Lemon grass leaf extract at 100mg/ml, produced inhibition zone diameters of 13mm and 14mm against the typed *S. aureus* strain and the test *S. aureus* respectively while at 50mg/ml, it produced an inhibition zone of 11mg/ml against *S. aureus* ATCC 6538 only (Table 2).

Table 1: Antimicrobial activity of cold, hot and ethanolic extracts of fresh *M. paradisiaca* and *C. citratus* leaves

Leaf extract		Zone of inhibition (mm) *S. aureus* ATCC 6538 Concentration (mg/ml)			*S. aureus* (test)		
		100	50	25	100	50	25
	a	0	0	0	0	0	0
Cold							
	b	0	0	0	0	0	0
	a	0	0	0	0	0	0
Hot							
	b	0	0	0	0	0	0
	a	22	0	0	26	0	0
Ethanol							
	b	0	0	0	0	0	0

a = Banana (*M. paradisiaca*) b = Lemongrass (*C. citratus*)

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Table 2: Antimicrobial activity of cold, hot and ethanolic extracts of dried *M. paradisiaca* and *C. citratus* leaves

Leaf extract		Zone of inhibition (mm) *S. aureus* ATCC 6538 Concentration (mg/ml)			*S. aureus* (test)		
		100	50	25	100	50	25
	a	0	0	0	0	0	0
Cold							
	b	0	0	0	0	0	0
	a	0	0	0	0	0	0
Hot							
	b	0	0	0	0	0	0
	a	0	0	0	0	0	0
Ethanol							
	b	13	11	0	14	0	0

a = Banana (*M. paradisiaca*) b = Lemongrass (*C. citratus*)

DISCUSSION

The reason why the dried *Musa* leaf extracts showed no antimicrobial activity against the test bacteria may be due to the varying degree of solubility of the active constituents in the extracting solvents as a result of heat associated with the soxhlet extraction method which could have inactivated some thermolabile components of the extracts (Nakwaide *et al.*, 2015; Abah and Egwari, 2011). The fact that ethanolic extracts of fresh Banana leaves inhibited the growth of *S. aureus* isolates but not the ethanolic extracts of fresh Lemon grass leaves apparently shows that the extracting alcohol may not have any inhibitory effect on *S. aureus*. This means that the fresh Banana leaves may contain some bioactive anti-staphylococcal substances (Nwankwo and Amaechi, 2013). It is possible that dried ethanolic extracts of *C. citratus* leaves showed activity against *S. aureus* because a higher proportion of the plant bioactive constituents were alcohol soluble (Asaolu *et al.*, 2009; Naikwade *et al.*, 2014). This study,

resolutely established the efficacy of ethanolic extracts of dried Lemon leaves and ethanolic extracts of fresh Banana leaves against *S. aureus*. This indicates that the plants produce compounds that are able to inhibit the growth of *Staphylococcus* using ethanol as a solvent for extraction. However, there is need for further investigations to be performed using different methods, extraction solvents and specific concentrations of the plants in order to determine their range of antibacterial activities.

Conflict of Interests

The authors declare no competing interest

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