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IN-VITRO ANTIBACTERIAL STUDIES OF FIVE MEDICINAL PLANTS AS ALTERNATIVE THERAPY FOR METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) INFECTIONS.

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ABSTRACT: The *In-vitro* antibacterial activities of five local medicinal plants against Methicillin Resistant *Staphyloccocus aureus* (MRSA) were investigated. The ethanol extracts of *Gnetum africanum* leaves proved to be the most effective against MRSA with the highest zone of inhibition of 30.5mm recorded. *Vernonia amygdalina*, *Ocimum gratissimum* and *Chromolaena odorata* leaves extracts showed diameter zones of inhibition of 16mm, 12.8mm and 15mm respectively. *Magnifera indica* root extracts also had activity with 15mm zone of inhibition. The Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) were also determined. *Chromolaena odorata, Mangifera indica, Vernonia amygdalina*, *Ocimum gratissimum* and *Gnetum africanum* had MIC values of 12.5mg/ml, 50mg/ml, 25mg/ml, 50mg/ml and 6.25mg/ml respectively. The MBC values of 50mg/ml, 100mg/ml, 50mg/ml, 200mg/ml and 25mg/ml were recorded for *Chromolaena odorata, Mangifera indica, Vernonia amygdalina*, *Ocimum gratissimum* and *Gnetum africanum* respectively. Generally *Gnetum africanum* was found to be the most active/potent against MRSA while *Ocimum gratissimum* was the least active.

KEY WORDS: *In-vitro*, Antibacterial, MRSA, medicinal plants, Alternative therapy, Infections

INTRODUCTION

Staphylococcus aureus is a facultatively anaerobic Gram positive Cocci which is responsible for a wide range of infectious diseases, such as microbial food poisoning, boils, abscesses, wound infection, pneumonia, toxic shock syndrome, skin infections etc (Bradley, 1991). There exist a number of antibiotic therapies available for the treatment of *S. aureus* infections. But of late, there is an increasing/rapidly developing problem of resistance to many antimicrobial agents (Wadlvogel, 2000). Of particular concern now and one of public health importance is the emergence of Methicillin-Resistant *Staphylococcus aureus* (MRSA). The term MRSA refers to those strains of *Staphylococcus aureus* bacterium that have acquired resistance to the antibiotics methicillin, oxacillin, nafcillin, cephalosporins, imipenem and or other beta-lactam antibiotics (Boyce, 1992).

Strains of Methicillin-Resistant *Staphylococcus aureus* (MRSA), which had been largely confined to hospitals and long-term care facilities, are emerging in the community. The changing epidemiology of MRSA bears striking similarity to the emergence of Penicillinase-mediated resistance in *S. aureus* decades ago (Boyce, 1998). Even though the origin of the emerging MRSA strains is not known, the prevalence of these strains in the community seems likely to increase substantially (Henry, 2001).

In the past two decades, the prevalence of MRSA strains has steadily increased in hospitals in the United States and abroad. National Nosocomial Infections Surveillance (NNIS) data collected by the CDC in the early to mid-1980s indicated that MRSA were limited mainly to relatively large urban medical centers at the rates of 5% to 10%.

Smaller, non referral centers were relatively free of MRSA, with prevalence rates well below 5%. By the 1990s, rates had increased to 20%. More recent surveillance data from NNIS indicates that rates have continued to rise, with prevalence of MRSA isolates from intensive care units approaching 50% by the end of 1998 (Layton *et al.,* 1995). Deaths have been recorded in children from infections of MRSA community acquired strains in the U.S in 1999 (CDC Report 1997-1999).

These reports of infection and colonization by strains of MRSA in children provide compelling evidence that MRSA strains, like Penicillinase-producing strains almost 30years ago, have gained a foothold in the community and are emerging as important outpatient pathogens. Based on experience with Penicillin-resistant strains, prevalence of MRSA among community isolates may be as high as 25% within the next decade (Wenzel *et al*., 1998). Once MRSA has become firmly established in a facility, it is rarely eliminated (Conterno *et al.,* 1998).

Approaches to the control of MRSA vary widely, primarily because studies establishing the efficacy of specific infection control measures are lacking (Goetz and Muder, 1992). A variety of control measures have been reported and many of these reports cite beneficial results. It should be emphasized, however, that the efficacy of most measures used for surveillance prevention and control of MRSA has not been established in controlled studies (Bradley, 1999).

In light of this recent trend in the emergence of multi-drug resistant bacteria posing a challenge for the treatment of infections, the need to discover new antimicrobial substances for use in combating such organisms become pertinent (Tsuchiya *et al*., 1996). Current research on natural molecules and products primarily focuses on plants since they can be sourced more easily and selected on the basis of their ethno-medicinal use (Ramzi *et al*., 2005).

The present study is aimed at evaluating the antimicrobial potential of *Gnetum africanum*, *Ocimum gratissimum*, *Vernonia amygdalina*, *Chromolaena odorata* leaves and *Magnifera indica* roots, as alternative treatment of MRSA infections which have become a public health challenge.

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MATERIALS AND METHODS

Plant Materials

The plants samples of the *Gnetum africanum, Ocimum gratissimum, Vernonia amygdalina, Chromolaena odorata* leaves and the roots of *Mangifera indica* were brought from Ekeonuwa market Owerri, Imo state, Nigeria and were identified and authenticated by Dr. Mbagwu F.N a Taxonomist of the Department of Plant Science and Biotechnology of Evan Enwerem University, Owerri with voucher specimen deposited at the University herbarium. They were air-dried and then ground.

Extraction

Ground materials of the plants samples weighing 300g were Soxhlet extracted with ethanol and water in succession for 12 hours. The different extracts were each concentrated by evaporation until dry under vacuum. The residues of the plants extracted were resuspended in ethanol to a concentration of 60mg/ml and stored.

Organism

The organisms used were 30 clinical strains of Methicillin-Resistant *Staphylococcus aureus* (MRSA) obtained from the Federal Medical Centre Owerri, Imo state, Nigeria.

Evaluation of Antimicrobial Activity

The preliminary antimicrobial screenings of the plant extracts were carried out using the agar diffusion technique (Singleton, 1999).

Mueller Hinton agar plates were seeded with 0.1ml of 1/100 dilution of an overnight culture of the bacterial isolate and allowed to stand. A standard cork borer of 6mm diameter was used to cut uniform wells on the agar surface into which 0.2ml of the test solution of each extract (60mg/ml conc.) was added. The plates were incubated at 37ºC for 24 hours after which diameter of zone of inhibition were measured. Ethanol was included separately in each plate as solvent control.

Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentrations (MBC) of the Extracts

The MICs of the active plant samples were determined using the broth dilution method (Adeniyi *et al.,* 2000). The extracts were serially diluted using Mueller Hinton broth to final concentrations of 30mg/ml, 15mg/ml, 7.5mg/ml, g/ml and 3.75mg/ml. The tubes were then inoculated with 0.1ml suspension of the test organism and incubated at 37ºC for 24 hours. The MIC was taken as the lowest concentration that inhibited the growth of the organism. From the tubes with inhibited growth, 0.1ml of the content was plated out onto the surface of agar medium and then incubated for 24 hours at 37ºC. The MBC is taken as the lowest concentration without growth of organism on the agar plate.

RESULTS AND DISCUSSION

The ethanol crude extracts of the five medicinal plants from Nigeria were tested for their antibacterial activity against Methicillin-Resistant *Staphylococcus aureus* (MRSA), using the agar well diffusion method. The diameter zone of inhibition, minimum inhibitory concentration and minimum bactericidal concentrations for all the tested plant extracts are shown in Table 1. Ethanol alone was tested as solvent control and no activity was recorded. However, all the ethanol crude extracts from the different plant species showed varied antibacterial activities against the test organism- MRSA. This indicates that the activity recorded was due to the activity of the bioactive compound present in the plant crude extracts and not the solvent used in the extraction process.

From the results obtained, the extracts of *G. africanum* leaves showed the highest activity against MRSA with 30.5mm zone of inhibition. This was followed by *V. amygdalina* leaves extract with 16mm diameter zone of inhibition. *C. odorata* and *M. indica* had 15mm zone of inhibition each while *O. gratissimum* had a diameter zone of inhibition of 12.8mm.

To evaluate the effectiveness of the concentration of the ethanol crude extracts on the MRSA, the MIC and MBC tests were conducted using series of ethanol crude extract concentrations. The values as shown in Table 1 indicated that *Chromolaena odorata, Mangifera indica, Vernonia amygdalina*, *Ocimum gratissimum* and *Gnetum africanum* have MIC values of 12.5mg/ml, 50mg/ml, 25mg/ml, 50mg/ml and 6.25mg/ml respectively. The MBC values of 50mg/ml, 100mg/ml, 50mg/ml, 200mg/ml and 25mg/ml were recorded for *Chromolaena odorata, Mangifera indica, Vernonia amygdalina*, *Ocimum gratissimum* and *Gnetum africanum* respectively. Hence, *G.africanum* was the most active/potent against MRSA while *O. gratissimum* was the least active.

The antibacterial activities exhibited by these plant extracts reported here, corroborates the findings of other researchers who worked on the antimicrobial activities of these plants on various organisms. Nisit *et al*.(2005) reported that the ethanol extracts of the aerial parts of *C. odorata* showed antibacterial activity against *S. aureus* and *E. coli*. Irobi (1997) and Muskhazli *et al*.(2009) independently reported the antibacterial activity of the ethanol and methanol crude extracts of *C. odorata* against *S. aureus* and other bacteria. Similar activities have been reported for *M. indica* alcoholic extracts against *S. aureus* by Akinpelu and Onakoya, (2006) and Doughari and Manzara, (2008). Furthermore, Iwalokun *et al*.(2003) reported the anti-MRSA activity of *V. amygdalina* from Nigeria. Likewise, the plants, O. *gratissimum* and *G. africanum* have been reported to have anti *S. aureus* activities (Nweze and Eze, 2009). While these plant extracts possessed antibacterial activities, the control antibiotic-Streptomycin demonstrated higher antibacterial activity against the test organism.

The higher potency of the control antibiotic Streptomycin over the ethanol aqueous crude plant extracts could be attributed to the fact that conventional drugs and other pharmaceuticals are usually prepared from synthetic materials. By means of better manufacturing techniques and procedures enhancing purity, their antimicrobial/therapeutic effect will be better than the crude extracts as reported by Makanjuola *et al*. (2010). This observation is in agreement with Lenta *et al*. (2007), who reported that crude extracts are liable to contamination and deterioration which reduces their efficacy.

The antimicrobial effects demonstrated by the plant extracts may not be unconnected to the possession of

phytochemical compounds such as phenols and flavonoidsKnown to have antibacterial activities (Lino and Deogracious, 2006). These components may act by complexing with extra cellular and soluble proteins of microorganisms (Mellissa *et al*., 2005).

Demonstration of anti-MRSA activities by these plants is an indication that there is the possibility of sourcing for alternative antibiotic substances/compounds from these plants which may be free from the disadvantages of synthetic antibiotics. Further investigations of the test plants activities against a wide range of bacteria and fungi, identification and purification of their chemical constituents as well as their toxicological effects is being carried out with a view to developing novel drugs for human consumption.

Table 1: Antimicrobial Activities of the Plants crude ethanol extracts, the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) in mg/ml.

	Zone of Inhibition (mm)	MIC (mg/ml)	MBC (mg/ml)
C.odorata	15	12.5	50
M.indica	15	50	100
V.amygdalina	16	25	50
G.africanum	12.8	50	200
O.gratissimum	30.5	6.25	25
Streptomycin	21	0.5	15

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