DETERMINATION OF ANTI-*Salmonella typhi* ACTIVITY OF THE CRUDE EXTRACT OF *Allium sativum* (GARLIC)

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ABSTRACT

Garlic has been considered to be an excellent medical panacea and a natural antimicrobial drug that can be considered as an alternative form of treatment of pathogenic infections.The antimicrobial effects of fresh aqueous garlic extract (FAGE) and dried aqueous garlic extract (DAGE) against *Salmonella typhi* was studied. Antibacterial activity of FAGE and DAGE was characterised by inhibition zones of 5-29 mm and 5-19 mm respectively with FAGE giving a higher sensitivity against the tested isolate. *Salmonella typhi* was tested against some commercial antibiotics, susceptibility of *S. typhi* to these antibiotics was characterised by inhibition zones of 10-26mm against four antibiotics and no sensitivity or inhibition zones were recorded for six antibiotics. The partition principle of paper and TLC reveals that FAGE had more component ingredients than DAGE. The observations made in this study supports the use of garlic in health products and herbal remedies as a low cost intervention in the enhanced therapy against bacterial infections in Nigeria.

Keywords: ***Antibacterial Effect, Allium sativum*,Anti *-Salmonella typhi*.**

INTRODUCTION

The continous spread of multidrug-resistant pathogens has become a serious threat to public health and a major concern for infection control practitioners worldwide (Sanders and Sanders 1992).The therapeutic uses of varieties of antimicrobial agents have achieved little results in addition to increasing the cost of drug regimen. This scenario has paved way for the re-emergence of previously controlled diseases and has contributed substantially to the high frequency of opportunistic and chronic infection cases in developing countries (Guerrero *et al*., 2003, Ako-Nai *et al.,* 2003). The slow pace of newer antibiotic development coupled with the availability of fewer antifungal agents has provided the need to explore nature in search of phytotherapeutic agents with novel targets and mode of actions (Iwalokun *et al.,* 2004). The practice of complementary and alternative medicine is now on the increase in developing countries in response to the scientific basis for the efficacy of many plants used in folk medicine to treat infections (Vijaya and Amanthan 1997, Dilhuydy, 2003). These plants have been used extensively as alternatives to the control and treatment of infectious diseases. Research into the active compounds contained in some plants extract have provided considerable information in the world of chemotherapy and phytotherapeutic medicine. One of these plants used most intensively and widespread is garlic (Gongagul and Ayaz, 2010).

Garlic, a member of the Allium family (*Liliaceae*) has been used throughout history not only as food condiment but traditionally to treat a wide array of diseases namely; respiratory infections, ulcers, diarrhoea and skin infections. The use of garlic as a medicine and condiment predates written history. Although it has been used for its medicinal properties for thousands of years. Investigations into its mode of action are

relatively recent. It has a wide spectrum of action, not only is it antibacterial, antifungal and antiprotozoal, but it also has beneficial effects on the cardiovascular and immune systems (Harris *et al.,* 2001). Resurgence in the use of natural herbal alternatives has brought the use of medicinal plants to the forefront of pharmacological investigations and many new drugs are being discovered.

Garlic is believed to possess antimicrobial properties that can control a variety of infectious diseases. Reuter *et al.,* (1996) reported garlic as a plant with antibiotic, anticancer, antioxidant, immunomodulatory, anti-inflammatory, hypoglycemic and cardiovascular protecting effects. These broad spectrum of activity according to Block, (1985) has been atrtributed to the over 100 phytotherapeutic sulphur compounds present in varying concentrations in garlic, which are formed by crushing-induced metabolic action of the enzyme allicinase (a cysteine sulfoxide lyase) on the odorless amino acid allicin (Lawson *et al*., 1991). Variations in composition of garlic and genetic disparity among bacteria and fungi of the same or different species have been found responsible for the few inconsistencies in the antibacterial and antifungal properties of garlic extract (Kivanc and Kunduhoghu,1997; Lawson *et al*., 1991) necessitating the need for local antimicrobial testing of garlic.

Recent years have witnessed an upsurge in worldwide interest among scientific institutions, biological research and major pharmaceutical institutions in the use of medicinal plants part and/or its active ingredient in garlic health products and herbal remedies.The present study has investigated the antibacterial activity of fresh aqueous garlic extract (FAGE) and dried aqueous garlic extract (DAGE) against local multidrug-resistant bacteria *Salmonella typhi* while comparing its sensitivity against other synthetic antibiotics.

MATERIALS AND METHODS

SAMPLE COLLECTION AND PREPARATION

Fresh bulbs of garlic (*Allium sativum* Linn.) were purchased from local markets within Kaduna metropolis in Nigeria. The cloves were separated and peeled to obtain the edible portion. Twenty grams of the edible portion were washed thoroughly with distilled water and disinfected with 75% ethanol, chopped into pieces and crushed with mortar and pestle. It was homogenised in 500 ml of sterile distilled water in a blender. The homogenate was transfered into a beaker containing 200 ml absolute ethanol and allowed to stand for 24 hours. This was then filtered by passing it through a whattman No.1 filter paper to give a crude aqueous extract which was placed in a water bath at 50^{O}C to concentrate it.

The same was done to obtain the dried aqueous garlic extract. However, to obtain the dried aqueous extract, the fresh garlic was air dried for 5-7 days and ground into powder before extraction was carried out.

CULTURE COLLECTION

Pure culture of *Salmonella typhi* was obtained from clinical stool specimens and identified culturally, microscopically and biochemically. The pure isolates were then preserved on *Salmonella – Shigella* agar (S-S agar) and kept at 4^{O}C in a refrigerator.

ANTIBIOTIC SUSCEPTIBILITY TEST

Susceptibility to different antibiotics was tested using Muller-Hinton agar medium. Various commercial and widely used antibiotics were tested: Chloramphenicol (CHL), Spafloxacin (SP), Septrin (SXE), Ciprofloxacin (CPX), Amoxacillin (AMX), Augmentin (AUG), Gentamycin (GN), Perfloxacin (PEF), Tarivid (OFX), Streptomycin (S). Antibacterial activity was carried out by inoculating 10^6 CFU/ml onto the agar and this was spread over the agar surface and antibiotic discs were placed on the culture media. Susceptibility of *Salmonella typhi* isolate to these antibiotics were recorded after incubation for 24 hours at 37 ^{O}C.

ANTIBACTERIAL EFFECT OF GARLIC ON *Salmonella typhi*

Susceptibility of *Salmonella typhi* was determined by agar well diffusion technique using Muller-Hinton agar. 3mm diameter wells (four wells per plate) were prepared on agar containing 0.1ml of *Salmonella typhi* (10^8 cfu/ml 1 MacFarland Standard). Both fresh and dried aqueous garlic extract was diluted 1:10 and different concentrations (0.025-0.8mg) were added to the wells. After 24 hours at 37 ^{O}C, the inhibition zones were observed and recorded.

IDENTIFICATION OF ACTIVE INGREDIENTS USING CHROMATOGRAPHY

Thin layer and paper chromatography were used to identify the active ingredients of both the fresh and dried aqueous garlic extract using hexane and isoprapanol as chromatographic solvent in ratio 3:1 (75ml:25ml). Capillary spotter was used to discharge extract of garlic onto the silica gel plate on the chromatography paper at 2.5cm from their posterior end and left for some time, then placed in the TLC chamber. The plates were observed under ultra-violet rays after standing in the iodine tank (this detects amino acids and flavanoids). The retardation (Rf) values for each separated component of the extract was measured and HRf calculated.

RESULTS

Salmonella typhi was tested for resistance profiles to ten antibiotics. The antibiogram profiles of the isolate revealed resistance pattern and inhibition zone of diameter to four (4) out of the Ten (10) antibiotics used as summarized in Table I. Inhibition zones were 20, 26, 10 and 20 mm for Chloramphenicol, Ciprofloxacin, Gentamycin and Perfloxacin respectively. *Salmonella typhi* isolate was resistant to Six (6) of the tested antibiotics (Septrin, Spafloxacin, Amoxacillin, Augmentin, Tarivid and Streptomycin).

Antibacterial effect of garlic to *Salmonella typhi* was determined using various concentrations of Fresh aqueous garlic extract (FAGE) and dried aqueous garlic extract (DAGE). Antimicrobial activity was exhibited when garlic extract was added into agar well at concentration of 0.25-0.8mg. FAGE antibacterial efficacy was observed to reduce resistance of *Salmonella typhi* by far when compared to inhibition zones of diameter obtained with DAGE. At 0.25mg *Salmonella typhi* showed resistance to both extract. However, only DAGE showed no observed effect in a zone of inhibition of 0.5mg. Growth inhibition of *Salmonella typhi* isolate was observed to increase with increase in extract concentrations.

In the chromatography experiment, when filter paper was stained with different reagents (Tables III and IV), several spots with different colors and different Rf were obtained. These spots show that fresh aqueous extract (FAGE) had more active ingredients over dried aqueous extract (DAGE).

DISCUSSION

Garlic has been known for its pungent odor and has been used as food and medicine in many cultures for thousand of years (Braun and Cohen, 2005). Garlic has been known for ages to have anti-infective properties against a wide range of microorganisms (Iwalokun *et al.,* 2004). The present study has however, further demonstrated the antimicrobial potency of fresh and dried aqueous garlic extract (FAGE and DAGE) against local *Salmonella* isolate. *Salmonella* isolate tested in this study is responsible for diseases such as diarrhoea and bacteremia due to multidrug-resistant infections. The observed zones of growth of inhibition on *Salmonella typhi* were comparable to those elicited by Chloramphenicol, Ciprofloxacin and Perfloxacin showing that the isolates exhibited susceptibility. This indicates that aqueous garlic extract has a broad spectrum of antimicrobial activity and a potential therapeutic window (Iwalokun *et al.,* 2004). However, the FAGE exhibited a more effective susceptibility potency when compared with DAGE.

The sensitivity of *Salmonella* isolate to FAGE over DAGE also implies that there are more intrinsic biosubstances in fresh garlic extract over dried extract as indicated by the chromatogram results in Tables III and IV. It is also due to the action of these biological active ingredient of allicin which exhibits its antimicrobial activity mainly by immediate and total inhibition of RNA synthesis. Although DNA and protein synthesis are also partially inhibited suggesting that RNA is the primary target of allicin action (Feldberg *et al.,* 1988). Iwalokun *et al.,* (2004) consequently stated that these intrinsic biosubstances are naive to the various drug resistance factors of the isolate which include beta-lactamases expression, increased pyrrolidonyarlamidase activity, aminoglycoside-modifying enzymes and altered ribosomal binding (Cercenado *et al.,* 1996 and Paparaskevas *et al.,* 2000). Meanwhile the antimicrobial potency of garlic has been attributed to its ability to inhibit toxin production and expression of enzymes for pathogenesis (Iwalokun *et al.,* 2004).

Several studies including those of Kumar and Sharma (1982), Adetumbi *et al.,* (1986), Rees *et al.,* (1993) and Reuter *et al.,* (1996) had demonstrated the antibacterial potency of aqueous garlic extract against enteropathogens such as *Vibrio parahaemolysis, E. coli, Klesiella* spp*, Proteus* spp*.* and *S.aureus* and anticandidal effects against *Candida spp*.. The antimicrobial potency disparity of garlic has been attributed to the different concentrations of individually and synergistically active biosubtances in garlic preparations coupled with their interactions with sulfydryl agents in culture media. This phenomenom has been used to explain the stronger antimicrobial effect of allicin than garlic oil disulfides (O`Gara *et al.,* 2000). Meanwhile allicin and other diallylsulfide compounds have been found at different concentration in aqueous garlic extract determined by age and method of extract preparation (Lawson, 1996). It can be said also in this study, that the concentration at which FAGE showed growth inhibitions is in contrast with observed antimicrobial potency of DAGE, as FAGE displayed non-comparable values.

The reliability of the clinical interpretation of the observations made in this study may undoubtedly require further tolerability of garlic extract to other multi-drug resistant pathogens. However, the poor potency exhibited by some antibiotics used and DAGE may be connected with the structural cell wall barriers of the isolates which confers resistance. The lipid composition of cell wall has been found to have an influence on the permeability of hydrophobic and volatile bioactive substances in garlic (White *et al.,* 1998).

In conclusion, the results of this study have attempted to provide scentific justification for the use of garlic against multidrug-resistant Salmonella infection and/or complementary and alternative medicine practices with plant extracts including garlic as a means of decreasing the burden of drug resistance would be of clinical and public health importance.

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Table I. Antibiotics resistance profiles of *Salmonella typhi*

Antibiotics	Concentration(ug)	Sensitivity	Inhibition zone (mm)
Septrin	30	-	
NI Chloramphenicol	30	+	20
Spafloxacin	10	-	NI
Ciprofloxacin	10	+	26
Amoxacillin	30	-	NI
Augmentin	30	-	NI
Gentamycin	10	+	10
Perfloxacin	30	+	20
Tarivid	10	-	NI
Streptomycin	30	-	NI

NI= no inhibition , + sensitive, - not sensitve

Table 2. Antibacterial effect of garlic extract on *Salmonella typhi*

Method	Concentration(mg)	Inhibition zone diameter (mm)
Agar well diffusion	FAGE	DAGE
800	29	19
400	15	13
200	14	10
100	9	5
50	5	NI
25	NI	NI

NI= no inhibition

Table 3: Thin layer chromatography of FAGE on Whattman No.1 filter paper using Hexane / Isopropanol

No. of spots	Ferric Chloride	Solvent front	Distance of solute(cm)	Rf	HRf(%)
1	Brown	12.0	3.5	0.29	29
2	Brown	12.0	5.3	0.44	44
3	Brown	12.0	6.8	0.57	57
4	Brown	12.0	8.0	0.67	67
5	Brown	12.0	10.0	0.83	83
6	Brown	12.0	11.6	0.97	97

FAGE: fresh aqueous garlic extract

Table 4: Thin layer chromatography of DAGE on Whattman No.1 filter paper using Hexane / Isopropanol

No. of spots	Ferric Chloride	Solvent front	Distance of solute(cm)	Rf	HRf(%)
1	Brown	12.0	7.3	0.61	6
2	Brown	12.0	9.5	0.7	71
3	Brown	12.0	11.2	0.93	93

DAGE: dried aqueous garlic extract