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ANTIBACTERIAL EFFECT AND MINIMUM INHIBITORY CONCENTRATIONS OF GARLIC (*ALLIUM SATIVUM*) EXTRACTS ON SOME HUMAN PATHOGENS

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ABSTRACT

Effects of raw and boiled extracts of garlic on some human pathogenic bacteria: *Vibrio cholerae, Proteus mirabilis, Salmonella paratyphi B and C, Shigella dysenteriae, Pseudomonas aeruginosa* and *Staphylococcus aureus* were examined by agar diffusion method. Minimum inhibitory concentration (MIC) of crude garlic extract was determined using *P. aeruginosa* and *S. aureus* by tube dilution method. It was found that raw garlic extract inhibited the growth of all the microbes to varying degrees at 24 hr of incubation. Action of the extract appeared bacteriostatic on *V. cholerae*, *P. mirabilis*, *S. paratyphi* B and C and *S. aureus*. At 48 hr of incubation, it was observed that *P. aeruginosa* and *S. dysenteriae* did not resume growth in the presence of the extract. None of the pathogens was prevented from growing in the presence of boiled garlic extract. The minimum inhibitory concentrations of the crude garlic extract were 134 mg/ml and 161 mg/ml for *P. aeruginosa* and *S. aureus* respectively.

INTRODUCTION

Garlic (*Allium sativum*) is commonly used as a spice and in the treatment of some diseases. Some of the traditional uses of garlic include the cure of cold, flu, infectious ear aches, vaginal yeast infections and high blood pressure[1]. Modern researches have focused on the application of garlic in the treatment of heart diseases, cancer and antioxidant effect[2,3,1,4]. Certain studies have also shown that the plant possesses antimicrobial property. A long time before microbes were discovered, French priests of the middle ages were using garlic against bubonic plague now known to be a bacterial infection[4].

In the late 19th century, research studies on effect of the garlic plant on infections have intensified[5,1,6]. Antibacterial, antifungal and antiviral actions of garlic have been reported by Hughes and Lawson[7]. Garlic extract was reported to be active against the growth of *Escherichia coli*, *Pseudomonas aeruginosa*, *Aspergillus flavus*, *Bacillus cereus*, *Corynebacterium diphtheriae*, *Candida albicans*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Salmonella typhimurium* and *influenza virus*[8,9,10,11,4,12]. In a study carried out by Boboye *et al.*[12], it was reported that the extracts of garlic, onion (*Allium cepa*) and ginger (*Zingiber officinale*) were effective against the growth of *Klebsiella pneumoniae* amongst other bacteria tested. Garlic extract prevented the growth of *Trichophyton rubrum* and *Microsporium auduini*[11]. Garlic has antiparasitic action. Nork *et al.*[14] reported that garlic pulp completely suppressed the ability of *Trypanosma brucei* to cause African trypanosmiasis in mice.

In order to assess the ability of garlic extract to cure diseases caused by some commonly encountered bacteria in Nigeria, a survey of the effect of the spice on *V. cholerae*, *P. mirabilis*, *S. paratyphi* B and C, *S. dysenteriae*, *P. aeruginosa* and *S. aureus* was investigated. Minimum inhibitory concentrations were determined for *P. aeruginosa* and *S. aureus*.

MATERIALS AND METHODS

Sources of Materials

Fresh bulbs of garlic were obtained from "Oba" market in Akure, Ondo State, Nigeria. *V. cholerae*, *P. mirabilis*, *S. paratyphi* B and C, *S. dysenteriae*, *P. aeruginosa* and *S. aureus* were

provided by Federal Medical Centre, Owo, Ondo State and Institute of Medical Research, Yaba, Lagos State, Nigeria.

Preparation of Extracts

A blender was surface-sterilized by using absolute ethanol for 30 min, drained and allowed to dry. Raw extract was made by grinding 100 g of the garlic in 150 ml sterile distilled water. This was boiled for 20 min to obtain boiled extract. Crude extract was prepared by filtering the raw extract through a sterile sieve with pore size of 0.1 mm.

Agar Diffusion Test

An aliquot (0.5 ml) of 18 hr old nutrient broth culture (OD_{600}, 0.45) of each of the bacteria was spread on nutrient agar. An hole was bored into the agar with 14 mm cork borer and filled with the raw extract. Control plate contained sterile distilled water instead of the extract. Another control plate was prepared as the test plate but lacked the organism substituted with sterile nutrient broth. All plates were incubated at 37°C for 24 to 48 hr and observed for clear zone which indicates sensitivity of the test organisms to the garlic. Degree of sensitivity was determined by measuring the diameter in the zone of inhibition in millimetre. This procedure was carried out three times and repeated using boiled garlic extract.

Determination of Minimum Inhibitory Concentration

This test was carried out by tube dilution method with crude garlic extract using *P. aureginosa* and *S. aureus* selected to represent Gram positive and Gram negative bacteria. A 0.5 ml of 18 hr *P. aeruginosa* (20 x 10^7 cells) and *S. aureus* (26 x 10^7 cells) were added to 9.5 ml peptone water contained in test tube. Crude extract was added to

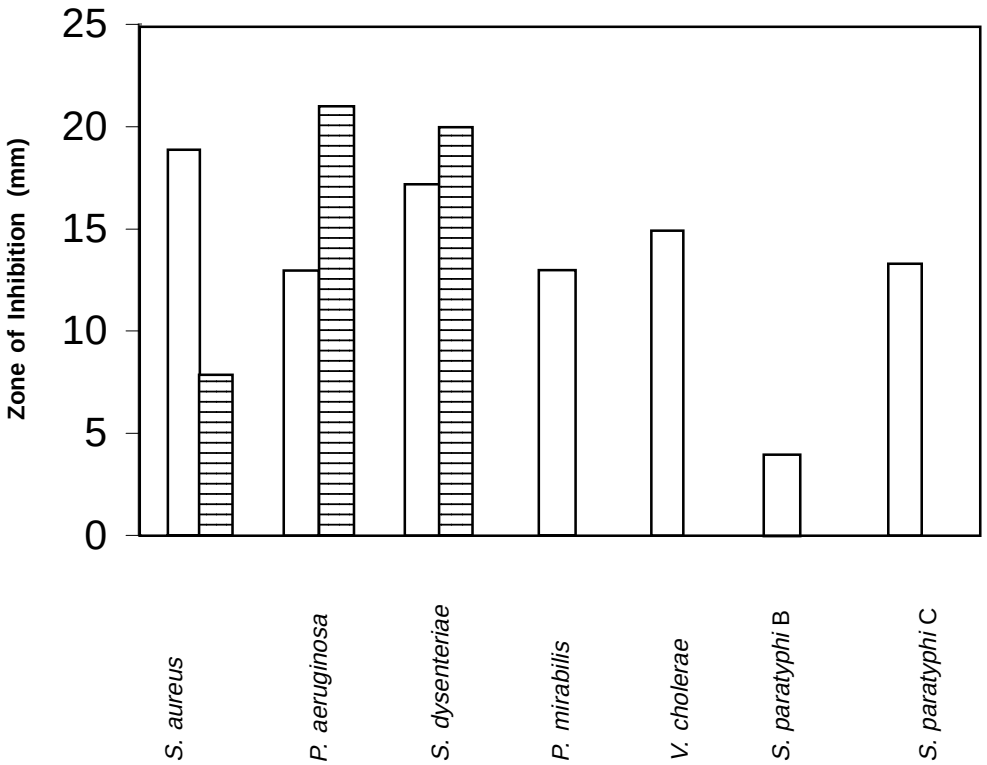

Fig. 1: Effect of raw garlic extract on some human pathogenic bacteria
: Inhibition to extract at 24 hr of incubation
=: Inhibition to extract at 48 hr of incubation

obtain varying final concentrations of 0.067, 0.134, 0.201, 0.268, 0.335, 0.402, 0.469 and 0.536 g/ml. Two sets of control tubes were prepared. A set contained nutrient broth substituted for the bacterium. Another set of control tubes lacked the extract replaced with distilled water. All tubes were incubated at 37°C for 24 hr. Transparency at the top of the liquid in the tube was recorded as inhibition. Aliquot (0.1 ml) of this liquid was inoculated into nutrient agar in order to confirm death of the bacterial cells. Garlic concentration in tube corresponding to where complete death of the organism was observed first was noted as the point where minimum inhibitory concentration started.

The above described procedure used for minimum inhibitory concentration (MIC) test was repeated to determine the specific MIC for each of the bacteria with final extract concentrations of 0.067, 0.0804, 0.094, 0.107, 0.121 and 0.134 g/ml for *P. aeruginosa* and 0.134, 0.147, 0.161, 0.174, 0.188 and 0.201 g/ml for *S. aureus*.

RESULTS AND DISCUSSION

At 24 hr of incubation, *S. aureus* appeared to be the most sensitive microbe to the raw garlic extract with the largest diameter (19 mm) in the zone of inhibition. This was followed by that of *S. dysenteriae* (17.3 mm), *V. cholerae* (15mm), *S. paratyphi* C (13.5 mm), *P. mirabilis* (13 mm), *P. aeruginosa* (13 mm) and *S. paratyphi* B which showed the least sensitivity with 4 mm diameter of growth inhibition (Fig. 1). At 48 hr of incubation, diameters of inhibition for all but two (*P. aeruginosa* and *S. dysenteriae*) of the microbes decreased to 8, 0, 0, 0 and 0 mm respectively. The zones of inhibition for the *P. aeruginosa* increased to 21 mm and that of *S. dysenteriae* to 20 mm. Zones of inhibition were completely covered with cells of *S. paratyphi* B and C, *P. mirabilis* and *V. cholerae*. This shows a resumption of full growth of these organisms.

Boiled garlic extract did not prevent the growth of any of the bacteria. On the other hand in the tube dilution MIC test, *P. aeruginosa* and *S. aureus* appeared susceptible to crude garlic extract starting from 134 mg/ml and 201 mg/ml. Data obtained from further MIC study showed that the minimum inhibitory concentrations for the two organisms were 134 mg/ml and 161 mg/ml respectively.

The findings in this work reveal that the raw garlic extract had inhibitory effects on the tested organisms. Resumed growth of some of the microbes observed at 48 hr of incubation suggests that the garlic has a bacteriostatic action on the bacteria. Garlic effect on *P. aeruginosa* and *S. dysenteriae* appeared bacteriocidal. In contrast, boiled garlic was ineffective against all the bacteria. In a clinical study, it was shown that cooking destroys the ability of garlic to produce allicin[4]. The result obtained in this work therefore suggests that the active constituent/s in the extract has/have been deformed, evaporated or destroyed by the applied heat. Principal ingredients in garlic are sulphur containing volatile oils which include allicine, ajoene, allyl sulphides and vinyldithins[15,4].

The MIC of the *P. aeruginosa* and *S. aureus* were close to each other with the former being most susceptible. These concentrations were higher than the standard value (30 mg/ml) recommended for MIC and those of many antibiotics such as gentamycin, tetracycline, erythromycin, ampicillin, tobramycin, kanamycin and chloramphenicol commonly used against these two organisms[16]. This could be attributed to the reason that the garlic is a plant containing other consitituents while the conventional antibiotics are purified substances synthesized by microorganisms wholly or partially from chemical compound which at low concentrations inhibit the growth of other microbes[17]. However, in a study carried out by Singh and Shukla[18], it was reported that garlic was more effective against clinical strains of *Staphylococcus*, *Escherichia*, *Proteus*, *Pseudomonas* and *Klebsiella* than the antibiotics (penicillin, ampicillin, deoxycycline, streptomycin and cephalexin) that they tested. Thus, based on the results obtained in this study and the fact that garlic is non toxic to human when consumed orally, garlic extract can be used to cure infectious diseases which are caused by the *P. aeruginosa*, *S. aureus* and similar microbial agents sensitive to the action of the plant. It is recommended that the extract should be purified and further studied.

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