Antibacterial Activity of Fresh Garlic Extract against Multi-drug Resistant Bacteria

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Abstract- ***Infection associated with multi-drug resistance bacteria are difficult to treat with standard antibiotics. Garlic is a powerful remedy to protect against infections of many bacteria, fungi and viruses. However, little is known about the potentials of fresh garlic extract (FGE) to improve the susceptibility of multi-drug resistant strains to antibiotics. Objectives: To investigate the antimicrobial activities of FGE of Sudanese and Chinese origin and the combination of antibiotics with FGE on methicillin resistant Staphylococcus aureus(MRSA), multi-drug resistant Pseudomonas aeruginosa, Escherichia coli, Klebsiella Pneumoniae and Proteus species and to evaluate the interactions between antibiotics and FGE. Materials and Methods: Clinical isolates were recovered from clinical specimens obtained from Soba University Hospital, Khartoum .This was carried out during the period from January to April 2017. The isolates consisted of MRSA ,P.aeruginosa ,E.coli , K. pneumonia and Proteus species ,10 each .Quality control for strains was obtained from National Public Health Laboratory (NPHL) Khartoum. This included S. aureus(ATCC®25923), P. aeruginosa(ATCC® 27853) and E.coli(ATCC®25922). .The 50 microorganisms were divided into five groups in a factorial design: control distilled water (D.W), Chinese GE, Sudanese Fresh garlic extract, antibiotics without fresh garlic extract and antibiotics with Sudanese and Chinese GE. Antibacterial activity was evaluated by measuring the diameter of inhibition zones according to performance standards for antimicrobial susceptibility testing of the Clinical and Laboratory Standards Institute (CLSI).***

Results: Atotal of 50 microorganisms were tested against Sudanese and Chinese Fresh garlic extract.Sudanese and Chinese Fresh garlic extract displayed evident inhibition properties against.

I. INTRODUCTION

In many developing countries, there are treasures of traditional medicine and natural narcotic traditions, based on the empirical knowledge of medicinal and toxic plants, acquired by grandparents and transmitted from generation to generation by oral tradition [1]. The use of medicinal plants has been given great importance because of its fewer side effects, improved patient tolerance, cheap cost, ease of access and a wide range of applications in many developed countries, 70 to 80% of the population used some alternative or complementary medicine in parallel with epidemiology, especially for the treatment and management of chronic diseases. 80% of the African population uses some form of traditional herbal medicine. In Ghana, about 70 percent of the population relies exclusively on traditional medicine for their health care [2,3,4]. There are many that are widely used in local forests and are used as a component of herbal medicines including species such as sativum (garlic) [5]. The history of garlic is more than 6000 years old and is an indigenous home in Central Asia. It has long been an essential element in the Mediterranean region, as well as frequent spices in Asia, Africa, and Europe[6].

Garlic is a perennial herb, it has a stem, long stem formation that reaches 2 - 3 feet in height. The plant has pink or purple flowers that thrive in the middle to late summer. The medically used part is the bulb. Previously classified in the family of Liliase (Liliase), Garlic is now a member of the family Aliasi and includes two basic types; hard neck and soft neck. The hard-necked garlic is characterized by hard, wooden centered stems that extend down to the basal plate at the bottom of the bulb. soft neck Garlic has a non-wood pseudo stem composed of overlapping leaf sheaths and rarely sent flower stalk unless climate conditions confirm [7].Garlic is effective due to its organic dissolved organic-soluble compounds [8]. Thiosulfins are primarily responsible for antibiotic activity because extracts of garlic-free thiosulfins normally lose their antimicrobial capacity [9]. Antibacterial drugs being widely used in clinical settings, many microorganisms, especially methicillin-resistant Staphylococcus aureus (MRSA), Pseudomonas aeruginosa ,have adapted to synthetic antibiotics and become highly resistant to these drugs over time [10,11]. Microorganisms with multi-drug resistance now cause thousands of deaths throughout the world each year [10-12]. Although some of these organisms can live harmlessly in humans and are carried in the nasal passage and on the skin, they can cause fatal infection in hospitals and nursing homes, where patients with open wounds, invasive devices and immunodeficiency are at higher risk of infection than healthy people [13]. Furthermore, resistance does make the infection more difficult to treat with standard antibiotics and thus more dangerous . Therefore, the continuing spread of multi-drug resistant strains and the increased abuse of antibiotics highlight the need for alternative agents. Garlic is also alleged to help prevent heart diseases

such as atherosclerosis, high cholesterol, and high blood pressure and certain types of cancer including stomach and colon cancer[6]. Garlic has definite antibiotic properties and is effective against a wide spectrum of bacteria, fungi and viruses [14,15]. In addition, the antimicrobial activities of garlic are linked to the presence of some bioactive compounds [16]. Moreover, many studies have demonstrated that garlic can be more effective as a broad-spectrum antibiotic compared with conventional antibiotics. However, most previous studies have only focused on the antimicrobial activities of garlic and garlic-derived organ sulfur compounds or the difference between garlic or garlic-derived organ sulfur compounds and standard antibiotics, while little is known about the potential of fresh garlic extract (FGE) to improve the susceptibility of multi-drug resistant strains to conventional antibiotics.The present study aimed to investigate the antimicrobial activities of Sudanese and Chinese fresh garlic extract and the combination of Sudanese and Chinese Fresh garlic extract and conventional antibiotics on common clinical strains, including Methicillin resistant staphylococcus aureusMRSA, multi-drug resistant Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae and Proteus species to evaluate the interactions between antibiotics and Chinese and Sudanese garlic extract.The study also aimed at comparing the activity of Sudanese and Chinese fresh garlic and their combination with antibiotics on the different bacterial isolates.

II. MATERIALS AND METHODS

A. Sudanese and Chinese Fresh Garlic Extracts

Garlic bulbs (Sudanese and Chinese) were obtained from the(Alanfal) local Market in Khartoum Sudan.Peeled garlic bulbs (100 g) from Chinese and Sudanese were blended in 50 mL sterile distilled water. The mixture was crushed finely using a juicer. The resulting paste was centrifuged at 3000 rmp for 30 minutes and the supernatant was then sterilized by a filter (0.2 µm pore size). The final concentration of Sudanese GE in aqueous solution was determined to be 59% (w/v) and Chinese GE 40.7 % (w/v) by subtracting the weight of the precipitate from the weight of the Original peeled garlic bulbs. The Chinese and Sudanese GE was stored in 1.5 mL Micro test tubes at -20°C until used.

B. Microbial Strains:

A total of 50 clinical isolates and three control strains were used. The clinical isolates were isolated from clinical specimens (wound swabs, ear swabs, blood culture, cerebrospinalfluid (csf), urine, abscess and pus samples) obtained from Soba Hospital and NPHL, Khartoum . Carried out during the period from January to April 2017. The isolates consisted of MRSA ,P.aeruginosa ,E.coli , K. pneumoniae and Proteus species, 10 each .Quality control for strains was obtained from central labs management, Khartoum. Which is : S.aureus(ATCC®25923), P. aeruginosa(ATCC® 27853) and E.coli(ATCC®25922). All isolates were identified using the biochemical reactions. It showed multiple antibiotic resistances, and had no apparent epidemiological connection. The isolated strains were then subculutred onto nutrient agar(NA) and stored at 2-8 °C until use. Purity of the organisms was checked at regular intervals by plating and staining. The 60 microorganisms were divided into five groups: controls (Distilled water), Sudanese garlic extract, Chinese garlic extract and antibiotics with garlic extract (Sudanese & Chinese).

C. Antimicrobial Susceptibility Testing:

This was done using:

1. Agar diffusion method:

The following antibiotic-containing paper disks were used: , cefoxitin (FOX) 30 µg, oxacillin (OX) 1µg, , cefotaxime (CTX) 30 µg , ceftriaxone (CRO) 30 µg, 10 µg ampicillin (AM) 10 µg, ceftazidime (CAZ) 30 µg, merpenem (MEM) 10 µg. McFarland turbidity (0.5) standard was used as standard for suspension of test organisms . Sensitivity testing using Kirby-Bauer disc diffusion technique as described in Clinical Laboratory Standards Institute (CLSI)[17].

The suspension was inoculated onto Mueller -Hinton agar (Five minutes after the agar absorbed the bacterial suspension; the antibiotic-containing paper disk on Mueller-Hinton agar plates and placed onto the surface of the inoculated plate (30 mm) with sterile forceps. Then 50 µL of Sudanese and Chinese GE was pipetted on the antibiotic-containing paper disk. The plates were left on a flat bench for 15 min after the paper disk absorbed the solution. Inoculated plates were then incubated at 37°C for 24 hour. The diameter of inhibition zone around each disk was measured to the standard values provided by CLSI[17].

2. *Cup- plate agar diffusion method*

Cup plate diffusion method was adopted with some minor modification to assess the antimicrobial activity of prepared extract 100 µL of bacterial suspension (standard and clinical isolate) were taken with automatic pipettes using sterile tips and added to twenty ml of molten Mueller Hinton media and mixed and poured in sterile plate .The media were allowed to set and solidify for minutes, make three wells using sterile Cork borer of 6 mm diameter. Alternated cups were filled with 50 µL of Chinese GE was

also Sudanese GE on tow separated wells Meanwhile, 50 µL of sterile distilled water was also pipetted on one well as the negative control. Allowed to diffuse at room temperature for15 min then the plate were incubated in incubator in upright position at 37°C for 24 hours. The diameter of resultant growth inhibition zones were measured in mm and the result were recorded.

C. Reproducibility and Statistics

All measurements were repeated two times and each strain was examined at least two separate times. Statistical analysis was conducted using the SPSS software version 16.0 (±mean).

III. RESULT

Atotal of 50 microoragnisms were tested against Sudanese and Chinese garlic extract. Table 1 showed the result obtained from the antimicrobial activity of oxacillin and cefoxitin with and without Sudanese and Chinese Fresh Garlic Extracts , against methicillin resistant Staphylococcus aureus. The activity of oxacillin and cefoxitin was dramatically enhanced by addition of Chinese FGE, and the synergism of OX and FOX with Chinese FGE yielded an obvious increase in the inhibition zones size(32.6 mm and 29.9 mm) respectively. Sudanese FGE alone was effective against MRSA, in the case of the combination with oxacillin and cefoxitin we found no significant increase in the inhibition zones size (10.7mm and 20.5mm)respectively.

Table 1:Mean zone of inhibition (mm) of Sudanese and Chinese FGE against MRSA

Chinese FGE without antibiotic	Sudanese FGE without antibiotic	OX wihout FGE	FOX without FGE	OX with Chinese FGE	FOX with Chinese FGE	OX with Sudanese FGE	FOX with Sudanese FGE
-	+	R	R	S	S	R	R
15.3	22.9	0.0	0.0	32.6	29.9	10.7	20.5

Abbreviations : OX= oxacillin, FOX= cefoxitin, FGE=Fresh Garlic extract (+) = Active ,(-) =less activity 0.0= No inhibition zone, R=resistant ,S=susceptible

Table 2 showed The antibacterial Susceptibility of Pseudomonas aeruginosa to meropenem and ceftazidime with and without Sudanese and Chinese FGE. The data showed that meropenem and ceftazidime with Chinese FGE could produce larger inhibition zones sized (22.4mm and 22mm) against P. aeruginosa , meropenem and ceftazidime with Sudanese FGE could produce smaller inhibition zones sized(10.5mm and 9.3mm)against P. aeruginosa compared with Sudanese FGE alone.

Table 2: Mean zone of inhibition (mm) of Sudanese and Chinese GE against P.aeruginosa

Chinese FGE without antibiotic	Sudanese FGE without antibiotic	MEM wihout FGE	CAZ without FGE	MEM with Chinese FGE	CAZ with Chinese FGE	MEM with Sudanese FGE	CAZ with Sudanese FGE
_	+	R	R	S	S	R	R
9.4	14.9	0.0	0.0	22.4	22.00	10.5	9.3

Abbreviations: MEM =meropenem,CAZ=ceftazidime, FGE=Fresh Garlic extract, (+) = Active (-)= less activity ,0.0=no inhibition zone, R=resistant,S=susceptible

figure 3 showed the result obtained the antimicrobial activity of cefotaxime,ceftriaxone and ampicillin with and without Sudanese and Chinese Fresh Garlic Extract Against Escherichia coli, Klebsiella Pneumoniae, Proteus species (ESBL). The activity of cefotaxime, ceftriaxone and ampicillin was dramatically enhanced by addition of Chinese GE, the synergism of cefotaxime ,ceftriaxone and ampicillin with Chinese GE against E.coli was (19.3mm),(19.2mm),(15.2mm) respectively while K.pneumoniae (21mm),(21.6mm),(16.9mm) respectively .and Proteus species (20.6mm),(18.5mm),(23.5mm) respectively. The activity of cefotaxime , ceftriaxone and ampicillin were insignificant by addition of Sudanese FGE, against E.coli was (5.4mm),(4.6mm),(5.3mm) respectively. While K.pneumoniae (9mm),(8.7mm),(11mm) respectively and Proteus species (1.8mm),(13mm),(12.5mm) respectively. Sudanese FGE alone was active against ESBL.

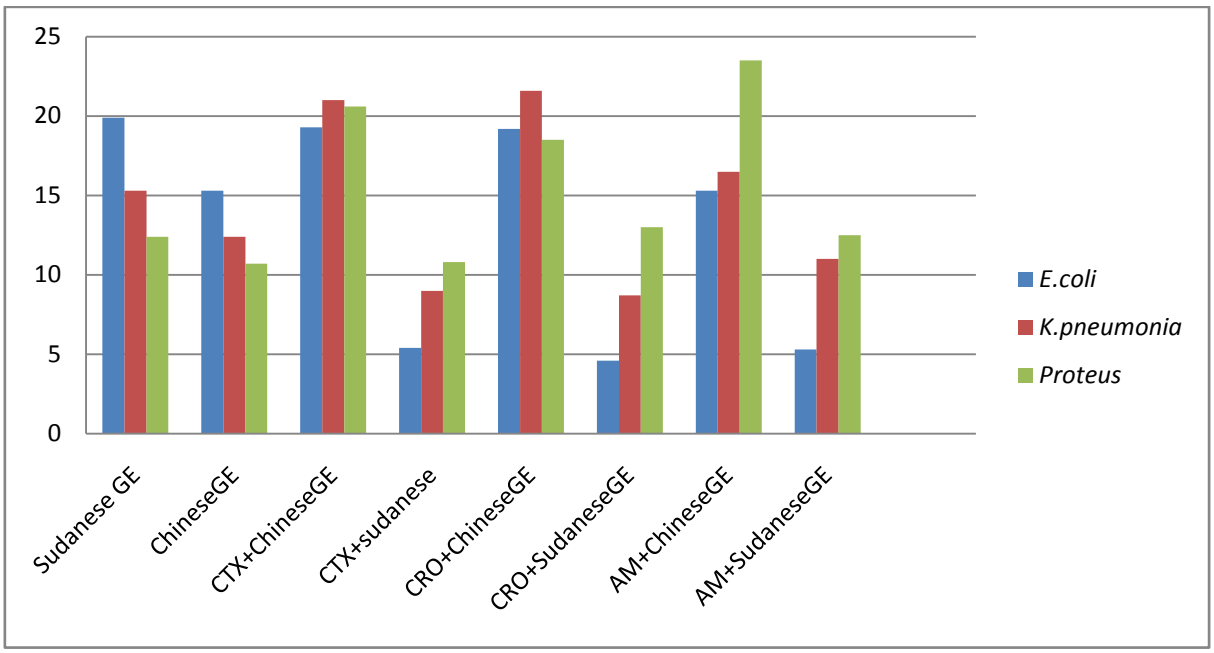

Fig 3:Mean zone of inhibition (mm) of Sudanese and Chinese FGE against ESBL producing pathogens.

Abbreviations :CTX= cefotaxime, CRO= ceftriaxone, AM= ampicillin.

IV. DISCUSSION

Methicillin-resistant Staphylococcus aureus is often considered to be as a "superbug" [18]. It was estimated that the number of MRSA infections in hospitals has increased significantly and the annual deaths from MRSA infections are even more than AIDS [20,21]. Garlic has been scientifically proven to be a powerful natural antibiotic against MRSA infections[18,19] . Ingredients in fresh garlic, other than illicit, have strong natural antibiotic effects[18] . Garlic extract, dially sulfide(DAS) and dially disulfide(DADS)provide powerful protective activity against MRSA by affecting the pathogen distribution and plasma levels of pro-inflammatory cytokines, endothelial injury-associated proteins, and coagulation and anti-coagulation factors as well as lipid oxidation levels, and by boosting the immune system. In this study, Chinese FGE produced a strong antibacterial effect on all MRSA resistance to standard antibiotics, cefoxitine and oxacillin, the inhibition zone size(synergism), Sudanese FGE against MRSA, in the case of the combination with cefoxitine and oxacillin produced no significant increase (antagonism). These finding are in accordance with previous studies [22]who studied the activity of fresh garlic.they found that FGE has inhibition properties against MRSA.

In our experiment ,the interaction effects of meropenem and ceftazidime with Chinese FGE was evaluated (table2) could produce larger inhibition zones size against P. aeruginosa compared with meropenem and ceftazidime without Chinese FGE . The Sudanese FGE alone produced large inhibition zones size against P.aeruginosa compared with Chinese FGE alone. These finding are not in accordance with previous studies [22]who studied the activity of fresh garlic extract . They found that FGE has weak inhibition properties against P.aeruginosa . Such difference could be attributed to the type of garlic been used.

In this study the data in (figure3) showed synergism of cefotaxime ,ceftriaxone and ampicillin . Chinese FGE was effective against K.pneumoniae, Proteus species and E.coli(ESBL) . Mean while we noticed that the Sudanese FGE alone gave a better results than the interaction with cefotaxime,ceftriaxone and ampicillin against those strains , our finding are not in accordance with previous studies[23] who studied heated garlic extract.They found that heated extracts of garlic and ginger had no antimicrobial activity against K.pneumonia Proteus species and E.coli. The action of fresh garlic is better than heated one.

V. CONCLUSIONS

Sudanese FGE without antibiotic displayed strong inhibition properties against MRSA, P.aeurginosa, E.coli, K.pneumonae and Proteus species .

However , the Chinese FGE without antibiotic show less inhibition growth of tested organisms ,but induced the activity when combined with antibiotics against those strains . We strongly Recommended the use of Sudanese FGE alone rather than Chinese FGE , but using Chinese FGE in combination with antibiotics (antibiotics

industry). Garlic is cheap, easy to find, has no side effect and can used in diet .

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