EUROPEAN ACADEMIC RESEARCH
Vol. IX, Issue 7/ October 2021

Impact Factor: 3.4546 (UIF)
DRJI Value: 5.9 (B+)

ISSN 2286-4822
www.euacademic.org

Assessment of in vitro antibacterial effects of Garlic and Ginger on Uropathogens isolated from Sudanese People in Kosti Teaching Hospital, Kosti, Sudan

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Abstract

Plants are the main source of medical care for a great proportion of the population. This study was done in microbiology laboratory of the faculty of medical laboratory sciences (University of El Imam El Mahdi) to study the antibacterial effect of alcohol extract of garlic (Allium sativum) and ginger (Zingiber officinale) against the isolated bacteria from different clinical specimens. Five different species of bacteria belonged to five different genera were isolated and these include the following organisms: Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Proteus mirabilis, and Staphylococcus aureus. The powder of plants was extracted with 70%

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alcohol to produce respective extracts. These extracts were screened for antibacterial activity by well diffusion and dilution methods. All tested bacterial organisms were most susceptible to the garlic and ginger methanolic extracts, the minimum inhibitory concentration (MIC) of garlic methanolic extract is 12.5% and (MIC) of ginger methanolic extract is 50%. The highest minimum inhibitory concentration were determined in garlic (Allium sativum) extract against all tested bacteria. In contrast ginger (Zingiber officinalae) extract showed partial antibacterial effect.

Key words: Garlic, Ginger, MIC, Uropathogens

INTRODUCTION

Plants are the main source of medical care for a great proportion of the population. Besides being cheap to produce, they are biodegradable and readily available [1]. The development of antimicrobial resistance (AMR) and emergence of new infectious disease create urgent need to discover novel, safe and effective antimicrobial compounds [2,3]. Plants derived compounds are likely to provide a valuable source of new antimicrobial agents. Additionally, several plants have ability to treat the multiple-drug resistant strains [2]. Garlic is consumed by almost every culture worldwide and it has a known medicinal properties, with a great of antibacterial activity [4]. Also, it has been used in herbal medicine for thousands of years [5,6]. Numerous studies have investigated the health benefits of garlic which include stimulation of the immune response, and the antimicrobial effects [6]. Moreover, the allicin derivative products (diallyl-disulfide, diallyl-trisulfide) that found in garlic essential oils have revealed a great antimicrobial activities [7].

Despite the existence of potent antimicrobial agents, resistant or multi-drug resistant strains are continuously emerging, imposing the need for a continuous search and development of new therapeutic strategies [8]. Such an approach can help suppress or considerably decrease the occurrence of drug resistance. Herbal compounds such as *garlic* could be the solution [3,9].

AMR inflicts high costs in the public health sectors of all countries [4]. The main cause was and continues to be a lack of public knowledge about antibiotics, resulting in their overuse despite recent stricter controls on their prescription and purchase worldwide [5, 10]. Human use/misuse of antibiotics has noticeably sited an unnatural selective force on bacteria, which has favored their accelerated evolutionary progression [11,12].

This study was aim to assess the in vitro antimicrobial effect of garlic extracts against bacteria isolates.

MATERIALS AND METHODS

This study was a cross sectional laboratory based study conducted at microbiology laboratory in faculty of medical laboratory sciences - University of El Imam El Mahdi during the period from March to July 2018. The study approval was provided by Ethic committee of the University of El Imam El Mahdi.

Bacterial isolates

In this work, five uropathogens were collected from positive culture of urine samples of UTI patents attended to Kosti teaching hospital in Kosti city of White Nile state. These isolates were *E.coli*, *P.aeurginosa*, *K.pneumoniae*, *P.mirablis* and *S.aureus*. Colour, shape, transparency and margin were examined and recorded as colony morphological characteristics according to [11,13]). Microscopic features were recorded for all isolates via Gram stain protocol. [12,14].

Preparation of garlic extract:

Fresh garlic bulbs was dried by exposure to sun light. subsequently, it was crushed and grinded to obtain a fine powder. 20 grams of fine powders were soaked in 100 ml of 70% methanol alcohol for 3 days at room temperature in universal bottle. throughout this period the bottle was shaken twice daily, after that was filtered using whattman filter paper N0 1. The filtered solution was dried in hot air oven at

250°C for 30 min to obtain powder which was collected, weighted and stored at 4°C pending further step.

Antibacterial activity of garlic extract

Antimicrobial efficacy of Garlic was assessed using Cup plate and broth Macro dilution method.

Cup plate method:

The bacterial cultures were refreshed on nutrient agar and microbial suspensions equivalent to 0.5 McFarland standards solution was prepared for every isolate. The agar well diffusion method was done on Muller Hinton Agar (MHA) medium for the evaluation of the antimicrobial activity of garlic methanolic extracts against the isolated pathogens. A sterile cotton tipped swab was used to inoculate test organism. A sterile cork borer was then used to make wells (4wells, 6mm diameter/well) on every MHA medium. Under aseptic conditions 100 µl of *garlic* extracts 100 %, 50 %, 25% and 12.5% were introduced into the wells (every concentration in different well). The plates were allowed to stand for 1hour in the refrigerator for diffusion of the extract to take place then incubated at 37 ^{o}C for 24 hrs. Methanol was used as negative control. Zone of inhibition were measured (in mm) and the mean were calculated.

Determination of minimum inhibitory concentration (MIC) of garlic extracts by Macro dilution method:

Dilution method was used to determine minimal inhibitory concentration (MIC). MIC of an antibacterial agent is the lowest concentration that inhibits growth of the isolated bacteria completely (absence of visible growth) [15].

Prepare the inoculums by making a direct broth suspension of isolated colonies selected from an agar plate (incubation not more than18-24 hour on non-selective medium). Then the suspension was adjusted to achieve turbidity equivalent to 0.5 McFarland turbidity standard. This resulted in a suspension containing approximately 1-2x10^{8} colony forming unit (CFU). For the macro broth dilution antibacterial assay, fivefold serial dilution of the extracts were prepared in tubes with distilled water as diluents from concentrations of 6.25%, 12.5%, 25%, 50%, and 100%. 0.5ml of each concentration was added to tubes containing one ml of test organisms. Then the tubes were incubated at 37°C, for 24 hour.

3. RESULTS

Five species of pathogenic bacteria including the following organisms: *E.coli*. *P. mirabilis*, *P. aeruginosa, S. typhimurium* and *S. aureus* were isolated form urine samples and subjected for full bacteriological identification methods, including gram stain reaction, morphological character and the biochemical tests. The susceptibility testing of suspension extracted of garlic and ginger were performed and zone of inhibition was determined for the different species as shown in (Tables - 1, 3), (MIC) was shown in Table 2 and 4 respectively.

The results obtained in this research showed that aqueous extract of garlic bulbs exhibit high antibacterial activity against *E. coli*, *P. mirabilis, S. aureus and S. aureus* (ATCC 25923) at different concentrations (100%, 50%, 25%) with inhibition zone (16,14,9), (18,14,12), (18,15,13) ,(17,15,13) respectively as in table-1. The minimum inhibitory concentration (MIC) which is the lowest concentration that inhibited the growth of the bacteria is at concentration 12.5% as in table-2. Aqueous extract of ginger and garlic revealed variable antibacterial activity against *P. mirabilis, P. aeruginosa*, *S. typhimurium, S. aureus*, and S. aureus (ATCC 25923), at different concentrations used (100%, 50%, 25%, 12.5%) with inhibition zone (35,30,27,15), (18,15,12), (15,8), (15,12),(14,12) respectively as in table-3. The minimum inhibitory concentration (MIC) is at 50%. as in table-4.

Table-1: The diameter of zone inhibition (mm) of bacteria for different concentrations of garlic extract:

Concentration of Garlic Extract	100%	50%	25%	12.5%
E. coli	16	14	9	-
P. aeruginosa	–	–	–	–
S. typhimurm	–	–	–	–
P. mirabilis	18	14	12	-
S. aureus	18	15	13	-
ATCC 25923	17	15	13	-

Table-2: The Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Garlic Extract:

Key: MIC = Minimum Inhibitory Concentration, MBC = Minimum Bacterial Concentration

Species	MIC
E.coli	12.5%
P.aeruginosa	
S.typhimurium	
P.mirabilis	
S.aureus	
ATCC 25923	12.5%

Table (3): The diameter of zone inhibition (mm) of bacteria for different concentrations of ginger extract:

	100%	50%	25%	12.5
E.coli	_	_	_	_
P.aeruginosa	18	15	12	_
S.typhimurium	15	8	_	_
p.mirabilis	35	30	27	15
S.aureus	15	12	_	_
ATCC 25923	14	12	-	-

Table-(4): The Minimum Inhibitory Concentration of Ginger Extract (MIC)

Organism	MIC
E.coli	50%
P.aeruginosa	100%
S.typhimurium	50%
P.mirabilis	50%
S.aureus	50%
ATCC 25923	50%

MIC=minimum inhibitory concentration

4. DISCUSSION

Infectious diseases are the major cause of death around the world and this problem is due to emerging of bacterial strains that had resistance to antibiotics. In order to minimize this problem, the new trends in microbiology is directed to find a cheap, natural, and available alternatives for the classical antibiotics, which can be performed by using plant extracts.

The results of present study showed that aqueous garlic extract bulbs exhibit high antibacterial activity against *E.coli, P.mirabilis, S,aureus* and *S.aureus* (ATCC 25923) at different concentrations (100%, 50%, 25%) with inhibition zone (16(17,25±0,75),14(14,5±0,5),9(11,75±1,25)), (18,14,12), (18,15,13) ,(17,15,13) respectively. The minimum inhibitory concentration (MIC) which is the lowest concentration that inhibited the growth of the bacteria is at concentration 12.5%. Aqueous extract of ginger and garlic revealed variable antibacterial activity against *P.mirabilis* , *P.aeruginosa*, *S.typhimurium*, *S.aureus*, and *S.aureus* (ATCC 25923), at concentrations used (100%, 50%, 25%, 12.5%) with inhibition zone (35,30,27,15), (18,15,12), (15,8), (15,12),(14,12) respectively. The minimum inhibitory concentration (MIC) is at 50%. The result of garlic extract was highly effective in suppress the growth of this bacterium, this findings is similar to [16] who found that the aqueous garlic extract was effective in inhibiting the growth of *P. mirabilis.*

According to activity of the ginger, the present study is disagreed with study that performed by [17] his findings showed that the potent antimicrobial activity of the ginger extract against the all tested bacterial pathogens, as the lowest zone of inhibition (8.0±1.73mm) against Escherichia coli, lower zone of inhibition (8.67±2.52mm) against Staphylococcus aureus compared to the Gram-negative bacteria.

5. CONCLUSION:

The results obtained in this research showed an explanation for the relatively therapeutic efficacy of plant materials (spices). Both garlic and ginger have antibacterial activity .Garlic and ginger have activity on both G+ve and G-ve bacteria . There are several advantages for the use of spices (that derived from plant origins) as or alternative medicine manifested by reduction the chance for developing antibiotic-resistant bacteria that resulted from the frequent use of antibiotics (misuse, abuse), beside decreasing the cost of treatment (drug administration) and also minimizes the development of adverse drug reaction

Recommendation:

We recommended for further in the future studies that should focus more on other advantages of spices especially the clinical applications in order to obtain low cost treatment and also prevention of recurrent infection.

It is beneficial to carry an invivo studies on antimicrobial effect of garlic and ginger against different type of microbial infections.

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