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In Vitro Studies of Garlic as Biological Control of Pathogens Isolated From Plums Fruit (Matunda Damu) in Dar Es Salaam

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Allium sativum is often used in different systems of medicines in ayurveda and Unani, plants contain chemicals which can inhibit the growth of microbes. *Moraxella cattarhalis*, *Klebsiella pneumonia, Pseudomonas aeruginosa* and *Proteus mirabilis,* pathogens were isolated from Plum fruit (Matunda damu), pathogens can spoil the fruits. We have conducted antibacterial activity of pathogens against Garlic extract and 7 antibiotics. All 4 pathogens were sensitive to Garlic extract. Pathogens showed sensitive to Azithromycin, cefotoxime ceftazidime and ciprofloxacin resistant to Gentamycin, tobramycin and ampicillin. Garlic extract showed equal strength as other antibiotics shown. Garlic, extracts represents alternative source of natural antimicrobial substances to control pathogens.

Keyword: Antibiotics, Antibacterial Activity, Plums, Allium Sativum

1. Introduction

Garlic (Allium sativum, Swahili-vitunguu) in the family Liliaceae is a perennial bulb-forming plant. It is known world-wide, and for several centuries, it has been used for dietary and medicinal purposes [1]. Garlic is an economically important plant [2]. It has antibacterial effect on gram positive and gram negative bacteria. Allicin exhibits its antimicrobial activity mainly by immediate and total inhibition of RNA synthesis, although DNA and protein syntheses are also partially inhibited, suggesting that RNA is the primary target of allicin action. The structural differences of the bacterial strains may also play a role in the bacterial susceptibility to garlic constituents [3].

Its uses have been inscribed on the walls of ancient Egyptian places of worship and pyramids. Its importance has often been highlighted Bacterial cultures in the scriptures [4].

A wide range of microorganisms including bacteria, fungi, protozoa and viruses have been shown to be sensitive to crushed garlic preparations. Allicin, thiosulfinates are active principles of freshly crushed garlic homogenates, has a variety of antimicrobial activities. Allicin in its pure form was found to exhibit antibacterial activity against a wide range of Gram-negative and Gram-positive bacteria. Inhibition of certain thiol-containing enzymes in the microorganisms by the rapid reaction of thiosulfinates with thiol groups was assumed to be the main mechanism involved in the antibiotic effect [5, 6].

Matunda damu is also known as Plums, is perishable sweet fruit containing high sugar and more than 85% water [7]. As water activity is high, isolates *Moraxella cattarhalis*, *Klebsiella*

pneumonia, Pseudomonas aeruginosa and *Proteus mirabilis* can spoil the fruits. These bacteria could enter into the fruits from environment or during processing. These gram negative bacteria can spoil the fruits and decrease shelf life.

Table 1: Antibacterial activity of different concentrations of aqueous garlic extract (AGE) by agar well method

Sl.No	Name of the bacteria	Diameter of Inhibition zone (mm) of different concentration of Garlic		
		100%	75%	50%
1	*Moraxella cattarhalis*	35	26	18
2	*Klebsiella pneumonia*	30	21	10
3	*Pseudomonas aeruginosa*	28	19	13
4	*Proteus mirabilis*	31	20	10

The aim in this study was to assess the antimicrobial activity of garlic extract compared with antibiotics against pathogenic bacteria isolated from Plums.

2.1 Materials And Methods

2.1.1 Collection of Plant Materials

The fresh forms of garlic (A. sativum) used in this study were collected on January 2013, from Kariako vegetables market, Dar es Salaam, Tanzania. The samples were brought to microbiology laboratory **St. Joseph University in Tanzania**.

2.1.2 Kirby-Bauer Disc Diffusion Method

Pathogen were identified by morphological and biochemical studies such as microscopic examination, Gram's staining, motility, catalase, coagulase, sugar fermentation test, biochemical tests using standard methods. Then these bacteria were screened for resistance by modified Kirby-Bauer disc diffusion method using 7 antibiotics discs were further used to find out the antibiotic sensitivity pattern of gram negative bacteria [8].

2.1.3 Antibiotic Sensitivity Testing

Antibiotic sensitivity testing was done using Kirby-Bauer method on Mueller Hinton Agar (HiMedia). 3.8 g of this agar was dispensed into a sterile conical flask. One hundred milliliter (100 mL) of distilled water was poured into the flask and stirred to dissolve the agar. The mixture was autoclaved and then poured into petri dishes. On gelling, the negative antibiotic sensitivity disk was introduced using sterile forceps and then incubated for 24 hours at 37 °C [8].

2.1.4 Antibiotic Discs Used

The disc diffusion was performed to test susceptibility of Salmonella and Shigella isolates using standard procedures [8]. Commercially available antibiotic discs for gram negative bacteria such as **ampicillin** (Amp) 10µg, **azithromycin** (Az) 15µg, **ciprofloxacin** (CIP) 10µg, **cefotoxime** (CTX) 30µg, **ceftazidime** (Cz) 30µg, **tobramycin** (TB) **10µg** G**entamycin** (GEN) 10µg were used. 10 hours old culture inoculated on the entire agar surface of each plate first in a horizontal direction and then in a vertical direction to ensure even distribution of the organisms. Antibiotic discs are placed after 5 min. to allow the agar surface to dry. The inoculated plates were incubated at 37 °C for 24-48 hr in an inverted position and the zone of inhibition was recorded. The zone of inhibition was expressed in terms of the Mean ± Standard Deviation by using four replicas and the results were tabulated.

Table 2: Antibiogram of known antibiotics against the test organisms.

Sl.No	Name of the bacteria	Maximum zone of inhibition in mm						
		AZ	CTX	GEN	CZ	TB	AMP	CIP
1	*Moraxella cattarhalis*	37	30	10	38	10	10	15
2	*Klebsiella pneumonia*	36	33	11	37	8	10	16
3	*Pseudomonas aeruginosa*	35	29	10	40	8	9	14
4	*Proteus mirabilis*	38	30	9	38	11	9	14

* Resistance below 12mm considered as bacteria is sensitive to antibiotics

2.1.5 Isolation of Microorganisms From Fruits

Surface swab cultures were taken from plums, aseptically brought to microbiology lab for analysis. Identified pathogens with gram test, biochemical test. Identified pathogens were *Moraxella cattarhalis, Klebsiella pneumonia, Pseudomonas aeruginosa,Proteus mirabilis* and *Bacillus subtilis.*

2.1.6 Aqueous Garlic Extract (Age) Preparation

The fresh forms of garlic were air-dried. The garlic bulbs were peeled, weighed (100 g), and cleaned garlic were taken and surface sterilized using ethanol. The ethanol was allowed to evaporate in a sterile laminar flow chamber. and the garlic was homogenized aseptically using a sterile mortar and pestle. The homogenized mixture was filtered through sterile cheesecloth. This extract was considered as the 100% concentration of the extract. The concentrations, 100%, 75%, 50% were made by diluting the concentrated extract with appropriate volumes of sterile distilled [9].

2.1.7 Antibacterial Activity Testing Using Agar Well Diffusion Assay

The selected strains of bacteria were inoculated into 10 mL of sterile nutrient broth, and incubated at 37 ^{o}C for 8 hours. The cultures were swabbed on the surface of sterile nutrient agar plates using a sterile cotton swab. Agar wells were prepared with the help of sterilized cork borer with 10 mm diameter. Using a micropipette, 100µl of different concentrations of garlic extracts (100%, 75% and 50%) were added to the wells in the plate. The plates were incubated in an upright position at 37 ^{o}C for 24 hours. The diameter of inhibition zones was measured in mm and the results were recorded. The inhibition zones with diameter less than 12 mm were considered as having no antibacterial activity [10].

3. Results and Discussion

We conducted antibacterial activity of different concentrations of aqueous garlic extract (AGE) by agar well method for 4 bacteria**.** Bacteria shown zone(mm) of inhibition for different concentrations of aqueous garlic extract (100%, 75% and 50%). Zone of inhibition for Moraxella cattarhalis was 35, 26 and 18mm for 100%,75% and 50% respectively. Zone of inhibition for *Klebsiella pneumonia* were 30,21 and 10mm for 100%, 75% and 50% respectively. Zone of inhibition for *Pseudomonas Aeruginosa* were 28,19 and 13mm for 100%, 75% and 50% respectively.

Zone of inhibition for *Proteus mirabilis* were 31,20 and 10mm for 100%, 75% and 50% respectively. Antibiotic sensitivity test conducted for 4 bacteria with 7 antibiotics.

Moraxella cattarhalis showed resistance to (10mm) ampicillin (Amp), (10) tobramycin (TB) and (10mm) Gentamycin (GEN) sensitive to (37mm) azithromycin(Az), (15mm) ciprofloxacin (CIP), (30mm) cefotoxime (CTX), (38mm) ceftazidime (Cz).

Klebsiella pneumonia showed resistance to (10mm) ampicillin (Amp), (8mm) tobramycin (TB) and (11mm) Gentamycin (GEN) sensitive to (36mm) azithromycin (Az), (18mm) ciprofloxacin (CIP), (33mm) cefotoxime (CTX), (37mm) ceftazidime (Cz).

Pseudomonas aeruginosa showed resistance to (9 mm) ampicillin (Amp), (8mm) tobramycin (TB) and (10mm) Gentamycin (GEN) sensitive to (35mm) azithromycin (Az), (15mm) ciprofloxacin (CIP), (29mm) cefotoxime (CTX), (40mm) ceftazidime (Cz).

Proteus mirabilis showed resistance to (9 mm) ampicillin (Amp), (11mm) to bramycin (TB) and (9mm) Gentamycin (GEN) sensitive to (38mm) azithromycin (Az), (14mm) ciprofloxacin (CIP), (30mm) cefotoxime (CTX), (38mm) ceftazidime (Cz).

Garlic can be used as a potent inhibitor of food pathogens. Foods contaminated with pathogens pose a potential danger to con sumer health. Use of garlic would increase the shelf life and decrease the possibilities of food poisoning and spoilage in processed foods [11]. In 2013 Dr. Nada KhazalKadhim Hindi showed similar results for *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Proteus mirabilis*. In 2010, Smyth AR showed inhibitory effect Garlic on *Pseudomonas aeruginosa*. Similar reports shown by B.A. Iwalokun in 2004 that Garlic extract was more effective against *Pseudomonas aeruginosa*. Rasheed MU in 2011 showed that *Moraxella cattarhalis* was sensitive to garlic extract. Meriga B etal in 2012 showed that Garlic can inhibit *Klebsiella pneumonia*.

4. Conclusion

Natural spices of garlic possess effective anti-bacterial activity against pathogens. Garlic has been shown to have almost miraculous preventive and therapeutic properties in the treatment of variety of diseases. It is a natural antibiotic. Frequent use of antibiotics causes the microbes to develop resistance against the drugs. Studies have shown that garlic has proven its ability to fight against bacteria that has developed resistance to antibiotics. The use of garlic along with other forms medication has been also known to enhance the effectiveness of these drugs.

Garlic extract solution can be used as spray to prevent the spoilage of fruits. Garlic extract enhance the quality and shelf life of fruits.

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