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The antimicrobial activities of ethanol and water extracts of garlic (*Allium sativum*) on selected pathogens

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Abstract

This study investigated the antimicrobial activity of garlic (*Allium sativum*) extract on *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Candida albicans* using disc diffusion method. Different extracts were obtained from the bulbs of garlic (water-soluble and ethanol-soluble extracts). There were zones of inhibitions around the discs embedded with the garlic extract. The result showed that all the extracts were found to be active against all the test organisms except *Klebsiella pneumonia* though with varying zones of inhibition. Ethanol extracts of the garlic have higher antimicrobial activity against the test organisms except *Klebsiella pneumonia* than water extract. This investigation indicates that garlic has antimicrobial effect thus can be used for chemotherapy.

Keywords: Inhibitory effect; Antimicrobial activity; Garlic; Water; Ethanol.

1. Introduction

A medicinal plant can be defined as any plant in which one or more of its parts contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Burkill, 2004)^[2]. The use of natural plant products is not new. In fact, it dates back to earliest history. An early school of thought about the medicinal uses of plants was called "the doctrine of signatures". It was believed that all plants existed for human use and that the proper use was clearly recognizable by the shape of the plant with bladder-like leaves which were good for ailments of the bladder-organ (Ward and Hetzel; 1999)^[21]. Plants produce many organic substances which have potential value in the treatment of diseases (Akinyemi *et al.*, 2006)^[1].

Sugar produced during the process of photosynthesis are converted by a series of well-defined processes, for example, phosphorylation, to all the other organic substances found in plants and in so doing incorporate nitrogen, sulphur to give proteins and glycerides together with many phenolic compounds, steroids, terpenes, alkaloids and other chemical substances. Medicinal properties of plants are normally dependent on the presence of certain phytochemical bases such as alkaloids, anthraquinones, cardiac glycosides, tannin and polyphenols (Gundiza, 1985)^[7].

Allium sativum (garlic) belongs to the family Liliaceae and genus *Allium*. It is a hardy perennial herb with narrow leaves. It has bulb-like root consisting of several bulbils (cloves) and the bulb is milky in colour. It has a strong smelling, powerful and penetrating odour and is used as a condiment in fish, meat preparations and in various fruit and vegetable preserves (Dutta, 1998)^[4]. *Allium sativum* (garlic) has been extensively found in India, China, Asia, Southern Europe, North America and the other Northern part of Nigeria. Garlic belongs to the genus *Allium* which is a general name for all substances which form allicins through enzymatic action. When fresh garlic is crushed, a characteristic odour is given-off which is mainly due to the disulphides compound allicin. Allins are odourless organic disulphides or their oxides and allicins are formed from allin by the action of enzyme allinase. Allicins have been reported to have antibiotic activity and it is considered to be responsible for most of garlic's pharmacological activities (Stoll and Seebeck, 2006)^[15]. The most important thing about garlic is that, it has not been known to contain any poison to animal and man as no cases have been reported.

Gomaa and Hashish, 2003 reported that *Allium sativum* (garlic) extract inhibited the growth of *Shigella dysenteriae*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*. Graham and Graham (1995) reported that mycelial growth produced by *Aspergillus parasiticus* was inhibited by garlic extract at a concentration of 0.3 - 0.4%. Galli *et al.* (1985)^[5] in their studies on the antimicrobial action of extracts of garlic (*Allium sativum*) reported the inhibitory action of garlic extract on gram positive and gram negative bacteria such as Bacilli and Clostridia, yeasts and moulds. Moulds were found to be more sensitive to the inhibitory effect than yeast.

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The growth of *Aspergillus niger* and *Candida albicans* was found to be inhibited by garlic extract as reported by Yoshida *et al.* (1996). Saleem Z. M. and Al-Delaimy (1982) [9] also reported the antimicrobial activity of garlic extract against *Bacillus cereus*, *Clostridium perfringens*, coliforms, *Escherichia coli*, Salmonella species and mould. The inhibition of growth of *Clostridium perfringens* by *Allium sativum* (garlic) extract was also reported by Tamer (2009) [18]. Extracts of *Allium sativum* (garlic) were found to be effective in diabetic treatment because of the ability of the active constituent to lower blood glucose and lipid levels. (Steiner and Lin, 1998) [13]. Ward *et al.* (1998) reported that garlic extract was effective in treatment of gastric cancer.

2. Materials and Methods

2.1. Collection of Plant Material

The fresh forms of *Allium sativum* (garlic bulbs) were purchased from Sabo market, Ogbomoso, Oyo state, Nigeria.

2.2. Collection of the Test Organisms

Local strains of the test organisms were collected from the Microbiology Laboratory of Ladoko Akintola University Teaching Hospital, Ogbomoso, Oyo State. The isolates included *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*. The test organisms were cultured on agar slants and stored in the refrigerator at 4 °C. Subcultures were made at two-week intervals.

2.3. Preparation of Garlic Extract

The husk of the garlic bulb was removed and the bulb was separated into cloves. The cloves of garlic were blended using a blender under aseptic conditions.

2.4. Preparation of Water Extract of *Allium sativum* (Garlic)

The extraction of the plant was done using the method of Opaleye and Olayemi (2002) [8]. Ten grammes (10g) of the grounded garlic was weighed on electronic weighing balance into sterilized conical flasks containing 100mls of distilled water and kept aseptically for four hours. The solution was passed through a funnel lined with filter paper to obtain the extract. The extract / filtrates were stored in a refrigerator of 4 °C as stock.

2.5. Preparation of Ethanol Extract of *Allium sativum* (Garlic)

Ten grams (10g) of the grounded powder of *Allium sativum* was weighed using electronic weighing balance and dispensed into sterile conical flasks. The sample was extracted with 100mls of 95% ethanol for four hours using soxhlet extractor. The ethanol was evaporated and the sample (garlic extract) collected. The extract was kept in the refrigerator at 4°C as stock. The extracts were prepared using serial dilution method. Three fold serial dilutions were made. 9 milliliters of sterile distilled water as dilute solution was pipetted into sterile test tube with suitable caps. One milliliter (1ml) of the stock solution (homogenate), that is, first serial dilution were removed and transferred into another test tube (third serial dilution). This procedure was carried out for ethanol and water extracts.

2.6. Preparation of Disc

The method employed was disc diffusion method as described by Stokes and Ridgway (1987) [16]. Discs of 5mm in diameter were punched out from a disc of Whatman filter paper. The discs were placed in petri-dishes, allowing a distance of 3mm between each disc and sterilized in a hot air oven at 160 °C for 1 hour. The discs were allowed to cool and 0.02ml of stock solution (1st serial dilution), second serial dilution and third serial dilution of garlic extract were pipetted onto different discs. The discs were dried by placing the sterile petri-dishes with lids slightly raised in a desiccator.

2.7. Testing for the Antimicrobial Activity

The culture medium was prepared according to manufacturer's specifications and sterilized by autoclaving at 121 °C for 15minutes and cooled to 45 °C and then poured into sterile petri-dishes and allowed to gel. The prepared plates were tested for sterility by incubating them at 37 °C overnight. The absence of growth on the plates indicated sterility. Using a sterile wire loop, subculture of the test organisms; *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* were uniformly spread over the surface of different sterile nutrient agar plates and allowed to dry while *Candida albicans* was uniformly spread over the surface of sterile Potato Dextrose agar plates and allowed to dry. The prepared disc containing 0.02ml of garlic extract was placed in the different petri-dishes seeded with the test organisms under aseptic condition and incubated for twenty-four hours and examined for zones of inhibition around the discs. The zones where present, were quantified by direct linear measurements of their diameters using transparent ruler. Discs containing 30µg chloramphenicol were placed in different petri-dishes seeded with *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and disc containing Nystatin was placed in petri-dishes seeded with *Candida albicans*.

3. Results

The sensitivity tests showed that *Allium sativum* (garlic) extract exhibited significant antimicrobial activity against *Staphylococcus aureus*, *Candida albicans*. *Pseudomonas aeruginosa* but it has no antimicrobial activity against *Klebsiella pneumoniae*. Garlic (*Allium sativum*) extract exhibited best antimicrobial inhibition on *Staphylococcus aureus* followed by *Candida albicans*.

The result showed that ethanol extract is more effective than water extract.

Table I: Respective Weight, Ph, Colour and Smell of Each Sample Extract

	Ethanol Extract of Garlic	Water Extract of Garlic
Weight Used	10g	10g
PH	6.8	7.0
Colour	Brownish	Colourless (cloudy)
Smell	True odour of garlic	True odour of garlic

3.1. Presentation of Data for Antimicrobial Activity

Table 2: Antibiogram of Ethanol Extract of Garlic (*Allium Sativum*) Against Test Micro-Organisms

Test Organisms	Zones of inhibitions (mean values) in MM				
	Dilutions of Ethanol extract			Control	
	Undiluted	10 ⁻¹	10 ⁻²	30µg Chloramphenicol	Nystatin
<i>Staphylococcus aureus</i>	14	13	11	14	-
<i>Pseudomonas aeruginosa</i>	11	10	8	6	1
<i>Klebsiella Pneumonia</i>	0	0	0	16	-
<i>Candida albicans</i>	12	11	9	-	10

Table 3: Antibiograph of Water Extract of Garlic (*Allium Sativum*) Against Test Microorganism

Test Organisms	Zones of inhibitions (mean values) in MM				
	Dilutions of Ethanol extract			Control	
	Undiluted	10 ⁻¹	10 ⁻²	30µg Chloramphenicol	Nystatin
<i>Staphylococcus aureus</i>	13	12	11	14	-
<i>Pseudomonas Aeruginosa</i>	9	8	6	6	-
<i>Klebsiella Pneumonia</i>	0	0	0	16	-
<i>Candida albicans</i>	11	10	9	-	10

Diameter of filter paper disc used: 5mm

The diameters of the zones of inhibition are obtained after subtracting 5mm respectively.

4. Discussion

According to Cheesbrough (1984) an antimicrobial agent which the diameter of the zone of inhibition is above 3mm, the organism is said to be sensitive but if it is 2mm or less than that, it is resistant. Therefore, considering this and comparing the values obtained with that of the control values, (Chloramphenicol and Nystatin), the results presented in Table 2 and 3 showed that the ethanol and water extracts of garlic (*Allium Sativum*) have antimicrobial effect against *Staphylococcus aureus* with the diameter of the zones of inhibition for ethanol extract being 14mm, 13mm and 11 mm for the different dilutions used, for water extract 13mm, 12mm and 10mm for the different dilutions used. The extracts also have antimicrobial effect against *Pseudomonas aeruginosa* with the diameter of the zones of inhibition being 11mm, 10mm and 8mm for ethanol extract and 9mm, 8mm and 6mm for water extract for the different dilutions used. The extracts also have antimicrobial effect against *Candida albicans* with diameter of zone of inhibition being 12mm, 11mm and 9mm for ethanol extracts and 11mm, 10mm and 9mm for water extract for the different dilutions used.

The results showed that ethanol extract have higher antimicrobial activity against the test organisms except *Klebsiella pneumonia* than water extract. The zones of inhibition became reduced when two-fold serial dilution of the extract is carried out. The results obtained showed that crude garlic extract has a broad spectrum of activity since it is effective against *Staphylococcus aureus*, *Candida albicans*, *Pseudomonas aeruginosa*. The antimicrobial activity of these extracts may be attributed to the presence of allicins which has been established by Stoll and Seebeck (1981). Allicins have been reported by them to have antibiotic activity and it is considered to be responsible for most of garlic's antimicrobial activity. The results of the ethanol extract and water extract of garlic however, showed that the extracts do not have antimicrobial activity against *Klebsiella pneumonia*. The diameter of zones of inhibition being zero. The non-activity of the extracts on *Klebsiella pneumonia* may be as a result of the complexity of the cell-envelope of these organisms which reduces the penetration of the extracts into cytoplasm. *Staphylococcus aureus* is susceptible with 14mm, 13mm and 11mm for ethanol extract as diameters of zones of inhibition while *Pseudomonas aeruginosa* is least susceptible with diameters of zones of inhibition being 11mm, 10mm and 8mm

for ethanol extract and 9mm, 8mm and 6mm for the water extract. Moreover, *Klebsiella pneumonia* is not susceptible.

The organisms used in this experiment have been selected carefully because of their various roles in animal and human diseases and in the spoilage of food. *Staphylococcus aureus* is a pathogenic bacteria that is capable of causing pyogenic infections which could be mild or severe. *Candida albicans* is known to cause disease in the body natural opening like vagina. It is the commonest cause of candidiasis. *Pseudomonas aeruginosa* causes skin infections especially at wounds, burn sites and urinary infections. *Klebsiella pneumonia* causes chest infections and urinary infections. Also these organisms occurs in the contamination and spoilage of various foods and food product such as poultry, meat and other protein foods, fruit salad, cheese, milk and so on.

5. Conclusion

Garlic extract has antimicrobial activity and should be used for chemotherapy. The antimicrobial effect of garlic extract has been demonstrated by the inhibition of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*. In this age of adulterated drugs, garlic extracts may be an alternative to the conventional or commonly used antibiotics for the cure of infections caused by these organisms.

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