

Efficacy of *Allium sativum* (Garlic) Bulbs Extracts on Some Enteric (Pathogenic) Bacteria

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ABSTRACT: Ethanol and water extracts of *Allium sativum* (garlic) bulbs were made through cold extraction and then concentrated by refluxing in soxhlet apparatus. A crude (raw) extract of the bulbs was also obtained by blending and then using muslin cloth to squeeze the juice out. These extracts were tried at various concentrations on four species of enteric bacteria namely: *Salmonella typhi*, *Salmonella paratyphi*, *Pseudomonas aeruginosa* and *Klebsiella* to observe their efficacy on these pathogenic bacteria. Crude extract showed a highly positive result and the rest were negative. [New York Science Journal. 2009;2(6):24-28]. (ISSN: 1554-0200).

Key words: efficacy, *Allium sativum*, extracts, enteric, bacteria

INTRODUCTION

The majority of people in Nigeria and presumably Africa as a whole use plant based traditional medicines for their care. The Pharmaceutical potential of African medicinal plants is immense. Plants like Neem (*Azadirachta indica*), Sodom apple (*Calotropis spp.*), Papaw (*Carica papaya*), Mahogany (*Khaya seneegalensis*) and a host of other plants are used for the treatment of several illnesses like fever, pile, stomach ache and so on.

In Nigeria, traditional healers and remedies made from plants play an important role in the health of millions of people especially in the rural areas (Rukangira, 2001). If ratios were to be compared between traditional practitioners and university trained doctors being patronized by the Nigerian populace; it is sure that there will be a tilt in high numbers, towards the traditional healers. This consequently means that most of the populace are more exposed and disposed to taking traditional recipe as opposed to the Orthodox, refined medicine (Rukangira, 2001).

It is also a known fact that the Orthodox drugs are refined from extracts of many of these medicinal plants. This is why the traditional medicine has some success story. In all countries of the world, there exists traditional knowledge related to the health of humans and animals. The importance of traditional medicine as a source of primary health care was officially recognized by the World Health Organization (WHO) in the primary health care declaration of Alma Ata (1978) and has been globally addressed since 1976 by the traditional medicine programme of the WHO (Rukangira, 2001). The programme defined traditional medicine as: "the sum total of all the knowledge and practices, whether explicable or not, used in diagnosis, prevention and elimination of physical, mental or social imbalance and relying exclusive on practical experience and observation handed down from generation to generation, whether verbally or in writing" (Rukangira, 2001).

One disease aside malaria that has in recent times infected and affected people around our community is typhoid fever. Typhoid fever is said to be caused by bacteria of *Salmonella* species. There have been cases of prolonged sickness and even death due to this disease.

Common drugs like chloramphenicol, gentamicin and ampiclox have always been used for treatment of typhoid fever. However, there have been rising cases of resistance of the pathogen to these drugs. It therefore behooves us to develop new therapies to counter the resistivity of the pathogen to already developed drugs.

In rural community, a traditional recipe is being used for the treatment of diagnosed typhoid cases. The recipe is a mixture of extract from bulbs of garlic (*Allium sativum*) and leaves of *Moringa oleifera* plant. These are washed, pounded and a glass of water added to it. This is sieved to give the filtrate. A glass full of the freshly prepared filtrate is taken first thing, every morning for five days. This application has shown some (though unrecorded) success story.

TYPHOID FEVER

The dictionary defines typhoid fever as an infectious fever caused by bacteria, resulting in red spots on the chest and abdomen and severe irritation of the intestines (Soanes, 2001).

Typhoid is an enteric fever which occurs only in man and is caused by a few *Salmonella* species and has a worldwide occurrence. Its occurrence and spread is encouraged by poor sanitary conditions. Strains resistant to recommended antibiotics have appeared in several areas of the world. Multi-resistant strains have been reported from Asia, the middle East and Latin America (Benson, 1990).

DESCRIPTION AND HISTORY OF GARLIC PLANT

The name is of Anglo-Saxon origin, derived from gar (a spear) and lac (plant), referring to the shape of its leaves. It belongs to the *liliaceae* family and genus *Allium*, which has more than six hundred (600) species. Included in this family are onions, shallots, leeks and bunching onions.

Garlic is believed to have originated in Western China from around the Tien Shan Mountains to Kazakhstan and Kirgistan. Vvedensky proposed that garlic evolved from the wild species *Allium longicuspus* (Derrida, 2003). The spread of garlic probably was first to the old world and then to the new world. One herb that seems to be universally known is garlic. It is revered and despised; revered for its potent health benefits and despised for its odour. Garlic has been in use for so long that it is difficult to pinpoint its particular origin. It is known that garlic grew wild in Southwest Siberia and spread through Southern Europe down to Sicily (Derrida, 2003).

For its cultivation, it prefers a rich, moist, sandy soil that is somewhat alkaline and a sunny place. Each clove from a bulb of garlic may be planted separately to grow new bulbs. They are planted about two inches deep and six inches apart with a one foot space between rows, new bulbs may be dug up in early fall when the leaves begin to die. It is cultivated today in most parts of the world.

The leaves of garlic plant are long, narrow and flat like grass. The bulb (the only part eaten) is of a compound nature, consisting of many bulb lets, known as cloves, grouped together between the membranous scales and enclosed within a whitish or purplish skin, which holds them as in a sack. The flowers are placed at the end of a stalk rising directly from the bulb and are whitish, grouped together in globular head or umbel, with an enclosing type of leaf or spathe, and among them as small bulbils.

While garlic is primarily used as an herb to enhance many food dishes in various cultures, many compounds can be found in its bulbs. It contains vitamins A and C, Potassium, Phosphorus, Selenium and a number of amino acids (Derrida, 2003). Most important are the over 75 sulphur containing compounds including allicin (S-allyl-L-cysteine sulphoxide). If the bulbs are grounded or crushed alliin is transformed into allicin (diallyl-disulphide Soxide) in which the typical garlic is attributed. A broad spectrum of antibacterial properties is associated with allicin (Derrida, 2003). Properties of the bulb include: adaptogen, alterative, antibiotic, anticoagulant, antifungal antineoplastic, antispasmodic, blood purifier, diaphoretic, digestive, expectorant, febrifuge, rebeneficent and stimulant (Derrida, 2003).

Phytochemicals common to bulb, flower, leaf and shoot are beta-carotene, niacin, riboflavin, and thiamin. The bulb contains the following, gamma-L-glutamyl-methionine; 1,2-(prop-2-enyl)-disulphane; 1,2-epithiopropane 1,2-dimercaptocyclopentane; 1,3-dithiane; 1-hexanol; 1-methyl-1,2-(prop-2-enyl)-disulphane; 1-methyl-2-(prop-2-enyl)disulphane; 1-methyl-3-(prop-2-enyl)-tri-sulphane; 2,3,4-trithiapentane; 2,5-dimethyltetrahydrothiophene; 2-methyl-benzaldehyde; 2-propen-1-ol; 2-vinyl-4H-1,3-dithiin; 3,5-diethyl-1,2,4-trithiolane; 3-methyl-2-cyclopentane-1-thione; 3-vinyl-4H-1,2-dithiin; 4-methyl-5-vinylthiazole and many more (Derrida, 2003).

Garlic finds application in many areas today due to its high potency. Among the many areas are: Garlic (*Allium sativum*) serve as immune stimulator. It is able to stimulate the immune system's macrophages, white blood cells that destroy foreign organism. It also increases the activity of Helper cells, and can be used to treat upper respiratory viral infections because of its ability to clear mucus from lungs and help asthma patients (Derrida, 2003).

Garlic serves as a good natural antibiotic. It is effective against bacteria that may be resistant to other antibiotics, and it stimulates the lymphatic system to throw off waste material. Unlike other antibiotics, garlic does not destroy the body's natural flora instead; it has the ability to stimulate cell growth and activity, thus rejuvenating all body functions (Derrida, 2003). From research, Albert Schweitzer is reported to have used garlic when in Africa for treating amoebic dysentery and as an antiseptic in preventing infections. (Derrida, 2003). Garlic is known to be effective in inhibiting V bacterial growth and many different strains of mycobacterium, viruses, worms and fungi. A study with aqueous garlic extract demonstrated significant *in-vitro* inhibition of a number of drug-resistant bacterial strains, and promising *in-vivo* activity when tested against *Shigella flexneri* in rabbits. Other researchers screened 132 extracts of plants used in folk medicine, and reported that *Allium sativum* extract was among those exhibiting the most

potent antibacterial activity (Derrida, 2003). Noteworthy is the performance of garlic extract against *Staphylococcus aureus*. The *S. aureus* strain used was resistant to both ampicillin and tetracycline, but 100% garlic was able to produce zones of inhibitions. *Allium Sativum* does display antimicrobial properties effective against a wide spectrum of pathogens (Derrida, 2003).

MATERIALS AND METHODS

COLLECTION OF BACTERIA SAMPLES

Stock culture of four bacteria samples viz *Salmonella paratyphi*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were collected from microbiology laboratory of Federal University of Technology, Minna. These cultures were used for analysis.

MEDIA PREPARATION

All anhydrous media which include Nutrient agar, Selenite F', Triple sugar iron agar, sugars, methyl red — Voges proskauer media were prepared according to the manufacturer's instruction. They were mixed and dissolved in distilled water, made up to the required volume, in some deserved cases, heated up to boil and then sterilized and poured into Petri dishes / test tubes.

GRAM STAINING

This divides bacteria into two categories, depending on whether they can be decolourized with acetone, alcohol or aniline oil after staining with dye of crystal violet, and treating with iodine. Those that resist decolourization remain blue or violet in colour, and are designated Gram + (positive); while those that are decolourised are termed Gram — (negative) (Baker et al., 2001).

BIOCHEMICAL TESTS

Respective isolated colonies of the four test organisms were inoculated into Triple Sugar Iron (TSI) agar and nutrient broth. Further tests including methyl red, Voges proskauer (VP), Indole, catalase, oxidase and sugar fermentation were carried out to confirm the identity of these micro organisms.

EXTRACTION FROM FRESH GARLIC BULBS

50g of fresh, peeled garlic bulbs were weighed in two places. These weighed bulbs were washed separately in clean water and pounded differently in a washed, dry and clean mortar. First part of the pounded (crushed) bulbs was soaked in 50ml of distilled water and the second part in 50ml of 98% ethanol in conical flasks which were covered tightly and left to stand for 48 hours. They were differently filtered using clean muslin cloth. About 3/4 of the filtrates were kept in the oven to evaporate.

CRUDE EXTRACTION FROM GARLIC BULBS

Some peeled garlic bulbs were blended, placed in a clean muslin cloth and the juice squeezed into a conical flask.

RECONSTITUTION OF EXTRACTS

For the extracts evaporated, they were reconstituted in the following manners:

- a. 0.2g of extracts in ethanol were dissolved in 2ml of ethanol and 8ml of glycerol added.
- b. Various weights of the extracts were dissolved in the same 2ml of ethanol and 8ml of glycerol with the highest weight being 5g of extracts.
- c. 0.2g of extracts in water were dissolved in 10ml of distilled water. Also various weights were used with the maximum at 5g/10ml of distilled water.

METHOD OF APPLICATION OF EXTRACTS

Basically two methods of application were used with a third type meant to verify or attest to the result obtained from the two methods.

a. AGAR PLUG HOLE METHOD

Sterile, molten nutrient agar was poured into petri dishes and allowed to solidify. Using sterile swab sticks, the test organisms were inoculated on the media by spreading. Holes were then drilled into the media using sterile cork borer (Ogechukwu, 2003). Various quantities of the extracts were introduced into the holes using sterile syringes with needles. The plates were then incubated at 37°C for 24 hours. Zones of inhibition were determined by measuring with a ruler and the result recorded.

b. DISK DIFFUSION TECHNIQUE

Disks were prepared using punch and whatman filter paper. The disks were sterilized, soaked in various concentrations of the different extracts and dried aseptically. Sterilized molten nutrient agar (45°C) was poured into sterile petri dishes and allowed to solidify. The plates were inoculated with test organisms by spread plate technique. The prepared disks were immediately placed at reasonable distances from each other on the inoculated plates having been previously soaked in the extracts and dried. These were incubated at 37°C for 24 hours and the observations recorded.

c. AGAR DILUTION METHOD

The third method used was the agar dilution method. In this method, various quantities (concentrations) of the extracts were added to sterile petri dishes. Sterile molten nutrient agar was added to the petri dishes and the dishes swirled for the molten agar and the extracts to mix thoroughly. The media were allowed to solidify. The plates were then inoculated with the test organisms and incubated at 37°C for 24 hours, observed and recorded.

RESULTS

Results of the biochemical tests on the microorganisms to verify their identification were obtained as in the table (Table 1) below.

Table 1: Biochemical tests on microorganisms

Test Organism	Gram Reaction	shape	Motility	TS		Glucose	Sucrose	Manitol	Lactose	Methyl Red	Indole	Voges proskauer	Citrate	Oxidase	Catalase
				Butt	Production H ₂ S										
<i>Salmonella typhi</i>	-	R	+	A	+	A	-	A	-	+	-	-	V	-	+
<i>Salmonella paratyphi</i>	-	R	+	A G	-	A	-	AG	-	+	-	-	-	-	+
<i>Pseudomonas aeruginosa</i>	-	R	+	A	-	A	-	-	-	-	-	+	+	+	-
<i>Klebsiella pneumoniae</i>	-	R	-	A G	-	AG	AG	AG	AG	-	-	+	+	-	-

KEY:

R = Rod A = Acid - = Negative G = Gas + = Positive
 V = Variable AG= Acid and Gas

The result showed that all the extracts at their various concentrations did not have an effect on the four microorganisms except the crude garlic extract as seen on the table below.

Table 2: Result of Agar plug method

TEST ORGANISM	RG		GW		GE		CG		Concentration (ml)	ZONE OF INHIBITION (mm)
	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.2		
<i>Salmonella typhi</i>	NE	NE	NE	NE	NE	NE	12	16		
<i>Salmonella paratyphi</i>	NE	NE	NE	NE	NE	NE	13	19		
<i>Pseudomonas aeruginosa</i>	NE	NE	NE	NE	NE	NE	5	10		
<i>Klebsiella pneumoniae</i>	NE	NE	NE	NE	NE	NE	10	10		

KEY:

RG = Refluxed garlic extract GW = Cold garlic in water extract
GE = Cold garlic ethanol extract CG = Crude garlic extract.
NE = No effect mm = Millimetre ml = Mililitre.

Table 3: Result of disk diffusion technique

TEST ORGANISM	RG	GW	GE	CG
	<i>Salmonella typhi</i>	NE	NE	NE
<i>Salmonella paratyphi</i>	NE	NE	NE	2mm
<i>Pseudomonas aeruginosa</i>	NE	NE	NE	2mm
<i>Klebsiella pneumoniae</i>	NE	NE	NE	NE

Similar result was obtained for the agar dilution method. Plates of all the extracts except for crude garlic extract showed a significant growth of the three microbes. No growth was seen on all plates of crude garlic extracts for the four microbes.

DISCUSSION

The result of this study revealed that garlic has some antimicrobial effect. The crude garlic extract showed a significant inhibition zone for all the bacteria samples, the ineffectiveness of extracts in water and ethanol could be due to denaturing of active ingredients resulting from the heat applied while refluxing and evaporating. This agrees with the findings of Derrida (2003) who stated that the constituents in garlic are protein, volatile oil, vitamins and other compounds. Probably the most beneficial compound is allicin, which is made by the enzyme alliinase breaking down alum to allicin. This process is essential to garlic's potency. Heating or cooking garlic inactivates the enzymes (Derrida, 2003). Cold, extraction on the other hand may not have succeeded in bringing out the active ingredients from the bulbs.

Garlic bulb extract is confirmed to have antimicrobial effect on *Salmonella typhi*, *Salmonella paratyphi*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

RECOMMENDATION

More research work should be carried out on this plant *Allium sativum* (garlic) as regards its antimicrobial effects. However, particular attention should be paid to the methods of extraction that will extract out all the potent phytochemicals without denaturing them. The extracts should also be tried not only on enteric bacteria but on a variety of pathogens generally.

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