

## THE EFFICACY OF *ALLIUM SATIVUM* (GARLIC) ON *ENTAMOEBA HISTOLYTICA*

<sup>1</sup>Rwang, P. G., <sup>2</sup>Mercy, K. P., & <sup>1</sup>Anyebe, G. E.

<sup>1</sup>Federal University Lafia

<sup>2</sup>University of Jos

### ABSTRACT

*Allium sativum* is a very good source of Vitamin C and is widely used around the world for its pungent flavour. It is also used as a diaphoretic, diuretic, stimulant, anti-asthmatic, digestive disorder, fungal infection and for the treatment of gastro-intestinal diseases. The crude extracts of garlic from different parts of the world have shown to possess anti-parasitic and anti-microbial properties. *Entamoeba histolytica*, a parasite, is responsible for the major human gastro-intestinal diseases. The efficacy of *Allium sativum* (garlic) on *Entamoeba histolytica* was studied using isolated organism from stool sample of amoebic dysentery which was inoculated into Locke's egg Slant Media. Incubations were carried out and the slants examined after 48 hours of inoculation at 37°C. Different extracts-ethanolic, crude, aqueous and hot water were prepared and different concentrations of each of the extracts-100%, 80%, 60%, 40% and 20% made and tested against *Entamoeba histolytica*. The result showed that all the extracts were effective except hot water extract. The highest zone of inhibition was 35mm and 22mm in ethanolic and crude extracts respectively at 20% concentration followed by the aqueous extracts at 100% concentration.

**Keywords:** *Allium sativum*, *Entamoeba histolytica*, Stool, Extracts, Efficacy.

### INTRODUCTION

The use of higher plant and their extracts to treat infections is an age-old practice in Africa Medicine <sup>[1]</sup>. Traditional medical practice has been known for centuries in many parts of the world. Plant extracts were given singly or as concoctions for various ailments and they include citrus, garlic, ginger and others <sup>[2]</sup>.

*Allium sativum* commonly known as garlic is a very good source of Vitamin C and is widely used around the world for its pungent spicy flavour, that mellows and sweetens with cooking <sup>[3]</sup>. The most commonly used part of the garlic is its bulb. It is also used for its diaphoretic, diuretic, stimulant, anti-asthmatic, digestive disorder, fungal infections and for the treatment of gastro-intestinal diseases <sup>[4]</sup>. Reports indicated that garlic plays a role in the reduction of death caused by malignant diseases. Garlic constituents are shown to exhibit antitumor and cytotoxic action both in vitro and laboratory animals and also to possess anti-bacterial and anti-parasitic activities <sup>[5]</sup>.

*Entamoeba histolytica* is a parasite responsible for the major human gastro-intestinal diseases such as Entamoebiasis or amoebic dysentery and amoebic liver abscess <sup>[6]</sup>. Its infective stage is the cyst and its host is man. It gains entry into man through the mouth and is transmitted when mature cysts are ingested through contaminated food or water. In man, *E. histolytica* resides in the

colon and caecum and trophozoites are the pathogenic stage <sup>[6]</sup>. For amoebic dysentery, the trophozoites/amoebae ingest red-blood cells from damaged capillaries; while for amoebic liver abscess, the amoebae are carried to the liver in the portal circulation and form abscesses usually in the right lobe <sup>[6]</sup>.

## METHODOLOGY

### Collection of Garlic

*Allium sativa* (Garlic) was collected from Yelwa Tudu Market Bauchi, Bauchi Local Government Area of Bauchi State, Nigeria.

### Preparation of Garlic for Extraction

Mature garlic bulbs were collected and shade dried. The dried garlic bulbs were pounded to powder in a wooden mortar and stored in labeled containers.

### Extraction of Crude Garlic Extracts

Powdered garlic material are mixed with distilled water and ethanolic solution in separate containers of one litre Pyrex conical flask each at a ratio of 1:5 weight per volume (w/v) according to the procedure described by <sup>[7]</sup>. Both mixtures were separately allowed to stand for 48 hours at ambient temperature and the suspension was filtered through layers of tin clothes in Mesh gauge. The filtrate was then concentrated to dryness by exposure to air. Extract obtained were scraped and stored in a refrigerator at 4°C in labeled specimen bottles.

### Preparations of Media

#### Locke's egg Media

Locke's solution was prepared by dissolving 8.0g of sodium chloride, 0.2g of calcium chloride and potassium chloride, 0.01g of magnesium chloride, 2.0g of sodium phosphate di-basic, 0.4g of sodium bicarbonate and 0.3g of potassium phosphate monobasic in 1000mls of distilled water. The solution was autoclaved at 121°C for 15 minutes under a pressure of 1516/in<sup>2</sup> cooled to room temperature, filtered to remove precipitants and re-autoclaved to sterilize.

For the egg slants, fresh hen's egg was sterilized by flaming in 70% ethanol broken into a graduated cylinder. 12.5ml of Locke's solution was added per 45ml of egg. It was emulsified in a blender and placed under vacuum to draw out air bubbles. 5mls of the emulsified egg was added to culture tubes and auto-clave at 100°C for 10 minutes with the tubes slanted. The resulting egg slant was cooled to room temperature and overlaid with 6mls of Locke's solution. It was autoclaved again at 121°C for 15 minutes under a pressure of 1516/in<sup>2</sup>. The slant was cooled to room temperature and refrigerated.

### Isolation of Organism

The test organism was isolated from a stool sample of amoebic dysentery obtained from the general hospital Bayara. The stool sample was inoculated into Locke's egg slant media.

Incubation was carried out and the slant examined after 48 hours of inoculation at 37°C as suggested by <sup>[8]</sup>.

### Identification of Test Organism

A smear was made from the growth on the slant with few drop of eosin reagent added to it and view under the microscope using X40 objective lens.

### Testing of Extracts

5mls of each concentration of the various extracts were dispensed into 10mls of the media in separate plates and inoculated from the pure cultures. The slants were incubated at 30°C for 48 hours <sup>[8]</sup>. The zones of inhibition of each concentration were recorded.

## RESULTS

Table one show the effect of garlic extract on the test organism. All the extracts except hot water extract were effective on the test organism. Further experiment showed the zone of inhibition caused by the different concentration of garlic extracts on the organism. However the hot water extract did not cause any inhibition to the organism. The ethanolic extract caused the highest inhibition at the concentration of 20% and 40% with inhibitory zones of 35 and 24mm respectively. The crude extract also had an inhibitory zone of 20mm and 11mm at 100% and 80% concentration respectively (Table 2).

Table 1: Effect of *Allium sativum* (garlic) on test organism

Extract	Cyst of <i>Entamoeba histolytica</i>
Crude extract	--
Ethanolic extract	--
Hot water extracts	++
Aqueous extract	--

#### KEY

- - = No Growth  
+ + = Growth

Table 2: Zone of Inhibition of *Allium sativum* (Garlic) extracts on *Entamoeba histolytica*

Extract	Concentration (%)				
	100	80	60	40	20
Crude extract	+ +	+ +	06mm	10mm	22mm
Ethanolic extract	+ +	08mm	15mm	24mm	35mm
Hot water extracts	+ +	+ +	+ +	+ +	+ +
Aqueous extract	20mm	11mm	09mm	03mm	+ +

#### KEY

- - = No Growth  
+ + = Growth  
mm = Millimeter

Ethanol extract in table 2 had the highest zone of inhibition of 35mm at 20% concentration. One would wonder why 100% concentration of ethanol couldn't inhibit the test organism, what happened in the ethanolic extract can be explained by osmosis of water in a universal solvent and permeates cells. At 100% concentration of ethanol extracts, there was growth because the extract had a higher concentration, hence could not permeate the cell but at 40% and 20% concentrations. The high concentration had been reduced hence molecules of the extract can permeate the cell of the test organism and can cause inhabitations to its growth. The same applied to the crude extract. For aqueous extract, 100% concentration was a low concentration that can permeate the cell. As the concentration reduced, the inhibitory zone also reduced because of the osmosis. At 20% concentration, the cell of the test organism had a higher concentration than the 20% concentration of aqueous extract, hence molecules moved from low concentration of the extract to high concentration of the cell, which caused the cell to absorb the water hence growth.

## DISCUSSION AND CONCLUSION

The result obtained from this study indicates that all the extracts except hot water extract of garlic, exhibited a remarkable inhibitory activity on *Entamoeba histolytica* which agreed with the findings of [9]. Allicin, an important component of garlic and other constituents like ajoene, alliin and others are the constituents linked to garlic's inhibitory properties and these constituents get deactivated on contact with it. This is possibly why the hot water extract was none effective on the test organism in both table 1 and 2 [10].

The above findings justifies the use of plants in the treatment of protozoan and other infectious diseases which agree with findings of [5], [9] and [11].

The potency of garlic plant was evaluated using ethanol, aqueous, hot water and crude extracts by cold extraction method. The study revealed the efficiency of garlic extract on *Entamoeba histolytica*. According to this result, garlic can be used for the treatment of amoebic dysentery and amoebic liver abscess. Studies by other researchers showed that garlic is effective on both gram negative and gram positive bacteria and fungi [12].

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