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## *In vitro* inhibitory activity of garlic and ginger extracts on some respiratory tract isolates of gram-negative organisms

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**ABSTRACT:** Fresh rhizomes of Ginger and cloves Garlic were collected, air-dried at room temperature and extracted separately using ethanol as solvent of extraction. The extracts were tested for antimicrobial activity against respiratory tract isolates of *Pseudomonas aeruginosa*, *Morganella morganii*, *Providencia* specie, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Proteus vulgaris*. The antimicrobial activity of ginger and garlic extracts on test isolates was determined using disc diffusion method. The results of sensitivity tests indicated that ethanolic extracts of both ginger and garlic have in vitro inhibitory activity against all isolates of Gram-negative organisms tested and sensitivity of the isolates increases with increase in concentration. Ethanolic extract of Garlic showed greater in vitro inhibitory activity than that of Ginger against all isolates tested.

**Keywords:** Respiratory tract, Garlic, Ginger, Extracts, Clinical isolates

### Introduction

Ginger is a member of the family Zingiberaceae; a small family with more than 45 genera, and 800 species (Newell *et al*, 1996). Its scientific name is *Zingiber officinale* named by an English botanist William Roscoe (1753 – 1831) in 1807 (Foster, 2000). It is an erect perennial plant growing from one to three feet in height; its stem is surrounded by the sheathing bases of the two ranked leaves. A clublike spike of yellowish, purple lipped flowers has greenish yellow bracts which rarely flowers in cultivation (Tyler, 2002).

Ginger is truly a world domestic remedy. It is also used in India and other places like the ancient Chinese where the fresh and dried roots were considered distinct medicinal products. Fresh ginger has been used for cold-induced diseases, nausea, asthma, cough, colic, heart palpitation, swelling, dyspepsia, loss of appetite, and rheumatism, in short for the same purposes as in ancient china (Foster, 2000). In nineteenth century ginger serves as a popular remedy for cough and asthma when the juice of fresh ginger was mixed with a little juice of fresh garlic and honey (Foster, 2000). A paste of powdered dried ginger was applied to the temples to relieve headache and fresh ginger was mixed with a little honey, tapped off with a pinch of burnt peacock feathers to allay nausea. One modern government health guide suggests one to two tea spoons of ginger juice with honey as a cough suppressant (Tyler, 2002).

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Ginger has been well – known in European homes for almost a thousand years (Foster, 2000). Asian cultures have used it for centuries, indigenous group of the Caribbean island were quick to adopt it as a remedy after its introduction to Americans by Francisco de Mandoca. Warm steamy fumes of hot ginger tea are used as an inhalant to relieve head colds in Jamaica (Tyler, 2002).

Garlic (*Allium sativum*) belongs to the family Alliaceae. Its close relatives include the onion, shallot, and leek. It has been used throughout recorded history for both culinary and medicinal purposes. It has a characteristic pungent, hot, flavour that mellows and sweetens considerably with cooking. The head of garlic (the most commonly used plant part) comprises numerous discrete cloves whereas the leaves and stems are sometimes eaten, particularly while immature and tender.

Garlic has been used as medicine in many cultures for thousands of years, dating as far back as the time that the Egyptian pyramids. It is also claimed to help prevent heart diseases including atherosclerosis, high cholesterol, high blood pressure, and to improve the immune system as well as protection against cancer (Marryland, 2005).

A daily dose of 1ml/kg body weight of garlic extract for six months can result in significant reduction in oxidant (free radical) stress in the blood of patients with atherosclerosis and cholesterol circulating in the bloodstream. Garlic's ability to prevent these oxidation reactions may explain some of its beneficial effects in atherosclerotic cardiovascular diseases (Wikipedia, 2007).

The study was aimed at determining the in vitro antibacterial activity of garlic and ginger ethanolic extracts on the isolates of Gram-negative organisms with the view to finding alternative means of treating infections caused by them.

## **Materials and Methods**

### *Collection of plant materials*

The fresh forms of *Zingiber officinale* (Ginger) and *Allium sativum* used in this project research were collected on May 2007, from Kurmi market, Dala, local Government area Kano state. They were identified in the department of biological sciences, faculty of science Bayero University Kano. The fresh forms of these plants were made into pieces, air-dried and made into powdered forms using a clean pestle and a mortar of microbiology laboratory of the department.

### *The test organisms*

The test organisms were bacterial isolates obtained from pathology department of Muhammad Abdullahi Wase Specialist Hospital, Kano. They were isolated from sputa samples submitted by patients having suspected respiratory tract infections. The isolates were subjected to Gram's staining and other biochemical tests according to standard procedures and identified as *E. coli*, *Proteus*, *Pseudomonas*, *Klebsiella*, *Providencia* and *Morganella species*.

### *Extraction of plant material*

The extraction was carried out according to the method of Fatope *et al* (1993). In this, 20g of the powdered plant samples were percolated at room temperature with 400ml and 300ml 97% ethanol for *Z. officinale* and *A. Sativum* respectively in 400ml beakers (thus achieving 1:20 and 1:15 ratio respectively). The beakers were covered with foil paper, shaken and left to stand for 2 weeks with regular shaking. After two weeks the suspensions were filtered and the filtrates were concentrated using Rotary-evaporating machine at 40°C. The extracts were labeled accordingly and stored in the refrigerator for further analysis.

### *Preparation of sensitivity discs*

Discs of 6mm in diameter were punched out using whatman No. 1 filter paper with the aid of a paper punch and placed in Bijou bottles. The discs were then sterilized by autoclaving at 121°C for 15mins after which they were allowed to cool.

#### *Preparation of sensitivity discs with ethanolic extracts of Z. officinale and A. Sativum*

Stock solutions of these two plant ethanolic crude extracts (that were recovered) were prepared by dissolving 0.5g (i.e 50mg) of each of the two plant extracts in 5ml of Dimethyl sulphoxide (DMSO). Therefore, each stock solution had a concentration of 10,000mg/ml. from these stock four (4) different concentrations of each the plants extract were prepared. These are 25µg /ml, 50µg/ml, 100µg/ml, and 200µg/ml which finally yielded disc potencies of 0.25µg/disc, 0.5µg/disc, 1µg/disc and 2µg/disc respectively. This was followed by introducing 100 sterile discs into each concentration. The discs were allowed to absorb the solution and kept for further analysis. Each paper disc is capable of absorbing 0.01ml (Kirby-Bayer, 1996).

#### *Bioassay Procedure*

The bioassay was carried out to determine the antimicrobial activity of the ethanolic extracts of ginger (*Zingiber officinale*) and Garlic (*Allium sativum*) against the Gram negative organism isolated. The bioassay was carried out using the procedure described by Cheesbrough (2000). Using a sterile wire-loop 3-5 well isolated colonies of the test organism were touched and emulsified into about 3ml of physiological saline. Turbidity of the suspension of test organism was compared with Mcfarland turbidity standard. Using a sterile swab stick, the test organism was inoculated onto sterile prepared nutrient agar media. The inoculated plates were then allowed to stay for about 3-5 minutes for the surface of the agar to air-dry. Prepared discs of the four different concentrations (i.e. 25µg/ml, 50µg/ml, 100µg/ml and 200µg/ml) for each of the Ginger and garlic ethanolic extracts were then placed on the inoculated nutrient agar media. Within 30 minutes of discs application, the plates were inverted and incubated aerobically at 37°C for 24hrs. Ciprofloxacin was used as control. After overnight incubation, the plates were observed or examined for zones of inhibitions. The zones of inhibition were measured in mm using a plastic ruler. The end of inhibition is where the growth starts.

## **Results and Discussion**

After extraction procedure, the crude ethanolic extract of *Zingiber officinale* and *Allium sativum* were obtained, weighed, and their physical appearances recorded. *Z. officinale* recovered extract was found to be 4.30g, brown in colour, gummy in texture with pungent odour, while *A. sativum* recovered extract was found to be 3.00g, light yellow in colour, gummy texture with allicin odour.

The zone of inhibition produced by the extracts against the test organisms was recorded as a measure of antimicrobial activity of the plant materials. The ethanol extracts of ginger showed remarkable activity against all the test organisms with the highest activity on *E. coli* (35mm zone diameter) at 200µg/disc concentration. It is also worthy of notice that even notorious organism like *Pseudomonas* which used to be resistant to many conventional drugs is sensitive to ginger extract at concentration of 25 µg/disc. The least activity is however seen with *Proteus vulgaris* in which even the highest concentration yielded only 13mm zone of inhibition.

The ethanol extract of garlic on the other hand is also active against the test organisms with highest activity on *E. coli* (51mm) at 200µg/disc. It is also very active on *Pseudomonas* with the least activity seen against *Proteus vulgaris*. Taura et al (2004) reported the activity of ethanolic extracts of garlic on some gram negative bacteria.

#### *Conclusions and recommendations*

Ginger and garlic have great potentials for the development of antimicrobial drugs most especially for the treatment bacterial infections of the respiratory tract. It could be recommended that:

- a) Further research be carried out on the toxicity of these extracts to ascertain their safety for human consumption.

b) The extracts should also be tested against gram positive bacteria and fungi so that they can be better exploited.

Table 1: Sensitivity of the isolates to ethanolic extracts of ginger

| Isolates              | Disc potencies (µg) |    |     |     |
|-----------------------|---------------------|----|-----|-----|
|                       | 25                  | 50 | 100 | 200 |
| Isolates              | Zone diameter (mm)  |    |     |     |
| <i>M. morganii</i>    | 14                  | 15 | 16  | 17  |
| <i>P. aeruginosa</i>  | 21                  | 23 | 25  | 26  |
| <i>P. vulgaris</i>    | 0                   | 7  | 9   | 12  |
| <i>P. mirabilis</i>   | 7                   | 8  | 15  | 16  |
| <i>Providencia</i> sp | 15                  | 28 | 30  | 32  |
| <i>K. pneumoniae</i>  | 0                   | 18 | 20  | 22  |
| <i>E. coli</i>        | 18                  | 33 | 34  | 35  |

Table 2: Sensitivity of the isolates to ethanolic extracts of garlic

| Isolates              | Disc potencies (µg) |    |     |     |
|-----------------------|---------------------|----|-----|-----|
|                       | 25                  | 50 | 100 | 200 |
| Isolates              | Zone diameter (mm)  |    |     |     |
| <i>M. morganii</i>    | 17                  | 25 | 26  | 28  |
| <i>P. aeruginosa</i>  | 28                  | 30 | 32  | 34  |
| <i>P. vulgaris</i>    | 8                   | 9  | 11  | 12  |
| <i>P. mirabilis</i>   | 8                   | 14 | 16  | 18  |
| <i>Providencia</i> sp | 19                  | 21 | 22  | 24  |
| <i>K. pneumoniae</i>  | 22                  | 23 | 24  | 26  |
| <i>E. coli</i>        | 47                  | 48 | 49  | 51  |

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