

Studies On The Antibacterial Activities Of Medicinal Plants On Typhoid Fever Organism

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Abstract

The antibacterial effect of raw, aqueous and ethanolic extracts of *Allium sativum* (garlic), *Zingiber officinale* (ginger) and *Moringa oleifera* (drumstick tree) on *Salmonella typhi* isolate, was investigated using the agar well diffusion method. The result of the study showed that raw *Allium sativum* and ethanolic extracts of *Allium sativum* and *Zingiber officinale* have inhibitory activity against the test organism. The minimum inhibitory concentration (MIC) ranged from 0.05-0.1g/ml(ml/ml). The inhibition zone diameter produced by the combinations of raw *Allium sativum* and *Zingiber officinale* as well as their ethanolic extracts were not enhanced, compared with their single activities against the test organism. Further exploitation of these two plant materials is hereby emphasized.

INTRODUCTION

Typhoid fever has remained a major scourge affecting most developing nations such as Nigeria, besides malaria. Typhoid fever causes an estimated 16.6 million cases and 600,000 deaths worldwide each year (WHO, 2003). Consequent on the current emergence and re-emergence of resistant strains of most microorganisms, coupled with side effects of most conventional drugs, interest in the use of plant and plant products in the management of ailments is increasing. Indeed, nature has remained a veritable source of medicinal agents since ancient times (Babayi et al., 2004). Although the in vitro and in vivo properties of some medicinal plants to microbial pathogens have been widely reported (Gomaa and Hashish, 2003; Arora and Kaur, 1999; Iwalokun et al., 2004; Nwosu and Okafor, 1995), most are actually yet to receive any scientific backing. The present study was therefore designed to determine the in vitro antibacterial effect of garlic, ginger and drumstick against the typhoid fever organism.

MATERIALS AND METHODS

COLLECTION AND IDENTIFICATION OF PLANT MATERIALS

The plant materials including garlic (*Allium sativum*), ginger (*Zingiber officinale*) and drumstick (*Moringa oleifera*) were obtained from Abakaliki, Ebonyi State, Nigeria. They were authenticated by a taxonomist of the Department of Applied Biology, Ebonyi State University,

Abakaliki.

TEST ORGANISM

Pure culture of *Salmonella typhi* isolated from stool samples of food vendors was obtained from the Genetics and Molecular Biology Laboratory Department, National Institute of Medical Research (NIMR) Yaba Lagos, Nigeria. The isolates was preserved in *Salmonella-Shigella* agar (S-S agar) and kept at 4°C until use. Macroscopy, Microscopy and Biochemical tests (Cheesbrough, 2002) were used to authenticate the isolate.

PREPARATION AND EXTRACTION OF PLANT MATERIALS

FRESH PLANT EXTRACTION

The bulbs of garlic, rhizomes of ginger and leaves of drumstick were washed with distilled water. The outer coverings of garlic and ginger were removed using sharp knife disinfected with 75% ethanol. They were subsequently cut into small pieces and respectively crushed using pestle and mortar. About 2ml of juice were expressed respectively from each crushed plant material into sterile bijoux bottles using a sieve. The extractions were done two hours before the commencement of sensitivity test.

DRIED PLANT EXTRACTION

Washed garlic bulbs and ginger rhizomes were cut into small pieces using a sharp knife and dried under mild sunlight.

Also, washed drumstick leaves were dried at room temperature. They were respectively ground into powder using pestle and mortar.

Exactly 20g of each of the pulverized garlic, ginger and drumstick were respectively introduced into 100ml of cold distilled water, boiled distilled water (boiled for 15minutes) and 95% ethanol. They were constantly agitated at 1hour intervals for 6 hours and then left for 24hours. Subsequently, they were filtered into clean containers using Whatman's No. 1 filter paper. The filtrates were dried to powder using a water bath at 40oC.

ANTIBACTERIAL SCREENING

Preliminary Screening- The surfaces of Mueller Hinton agar, prepared according to the manufacturer's instruction, were seeded with pure culture of *Salmonella typhi* adjusted to 0.5 Macfarland standard (Cheesbrough, 2002). Wells of 6mm diameter were made on the sensitivity agar media using sterile cork borer. Exactly two drops (0.2ml) of 0.5g/ml of each cold, hot water and ethanolic extract and two drops (0.2ml) of each raw juice extract respectively were introduced into the wells. They were allowed to stand for 1hour for proper diffusion and then incubated at 37oC for 24hours.

Determination of Minimum inhibitory Concentration (MIC)- To determine the minimum inhibitory concentration (MIC) of the dried extracts, the following concentrations were prepared; 0.025g/ml, 0.05g/ml, 0.1g/ml, 0.2g/ml, 0.4g/ml, 0.6g/ml and 0.8g/ml. Also, similar concentrations of their raw juices were also prepared. As previously stated, the test organism adjusted to 0.5 MacFarland standard was inoculated on sensitivity agar plates. Wells were made using sterile cork borer and 2 drops of each extract concentration introduced into separate wells. The plates were labeled accordingly and allowed to stand for 1 hour for proper diffusion. They were incubated at 37oC for 24hours. Chloramphenicol (25mg/ml) served as positive control while distilled water served as negative control for each plate.

Determination of Sensitivity of Extract Combination- The highest concentration (0.8g/ml) of ethanol garlic and ginger extracts were combined in a sterile bijou bottle (0.2ml:0.2ml). Also, the highest concentration (0.8g/ml) of the raw garlic and ginger were similarly combined. The combinations were properly shaken and used to conduct the sensitivity test, following the procedure as already described.

RESULTS

PRELIMINARY SCREENING OF PLANT MATERIALS

The preliminary screening of the extracts showed that raw extract of garlic and ethanolic extracts of garlic and ginger separately produced inhibitory activity against the test organism. All the extracts of the drumstick did not produce evidence of inhibitory activity against the *Salmonella typhi* isolate.

DETERMINATION OF THE MINIMUM INHIBITORY CONCENTRATION (MIC)

The Minimum Inhibitory Concentration (MIC) of raw garlic against the test organism was 0.05ml/ml, while that of the ethanolic extracts of garlic and ginger were 0.1g/ml and 0.05g/ml respectively. (Tables 1 and 2).

Figure 1

Table 1: Sensitivity pattern of to raw garlic extract.

Extract Concentration(ml/ml)	Inhibition Zone Diameter (mm)
0.025	NI
0.05	5
0.1	7
0.2	9
0.4	15
0.6	17
0.8	20
Control (Chloramphenicol 25mg/ml)	30

Figure 2

Table 2 :Sensitivity pattern of to ethanolic extracts of garlic and ginger

Extract Concentration (g/ml)	Inhibition Zone Diameter (mm)	
	Garlic	Ginger
0.025	NI	NI
0.05	NI	5
0.1	6	7
0.2	8	9
0.4	11	11
0.6	12	14
0.8	14	17
Control (Chloramphenicol, 25mg/ml)		

KEY : NI - No Inhibition

Inhibitory Effect of Extract Combinations on Test Organism> The combination of raw garlic and ginger did not produce enhanced inhibition zone diameter (IZD) compared with their individual single effects on the test organism. The same was the case with the ethanolic garlic and ginger combination (Table 3).

Figure 3

Table 3 : Sensitivity pattern of to extract combinations

Extract combination (0.8g/ml)	Inhibition Zone Diameter(mm)
Garlic and Ginger (raw extracts)	20
Garlic and Ginger (Ethanol extract)	17
Control (Chloramphenicol, 25mg/ml)	30

DISCUSSION

The result of this study showed that of all the raw extracts screened, only that of garlic exhibited inhibitory activity against the test organism. This is in conformity with the work of Gomaa and Hashish (2003), in which the inhibitory property of fresh garlic on the growth of some microorganisms including *S. typhi* was reported. They discovered that water extract of garlic produced higher antimicrobial reduction than the fresh ones. In the present study however, the aqueous extracts of garlic did not show any inhibitory property against the test organism. Although the reason for this variation is not very obvious, the probable differentiation in the garlic species and microbial strain, could be responsible. The antimicrobial activity of garlic extract against *S. typhi* has also been reported by other workers (Iwlokun et al., 2004; Arora and Kaur, 1999; Ekwenze and Elegalam, 2005).

The result of the present investigation emphasizes the usefulness of *Allium sativum* (garlic) in the treatment of diseases and the need to enhance its exploitation in this regard. This is particularly of urgent interest considering the rate of multi-drug resistance strains of organisms including *S. typhi* currently emerging world-wide (Prescott et al., 2005) .Also observed in this work was that only the ethanolic extract of ginger was able to produce inhibitory activity against the test organism. In addition to highlighting the importance of extraction solvents, it also adds ginger to the list of potential plant materials possessing inhibitory property against typhoid fever organism. In fact, the ethanol extract of ginger at 0.8g/ml concentration produced higher inhibition zone diameter (IZD) than the garlic extract (Table 2). The inhibitory property of ginger against *S. typhi*, *E. coli* and *B. subtilis* has been demonstrated by Azu and Onyeagba (2007).

Although the combination of the two ethanolic extracts at 0.8g/ml could not produce enhanced IZD, it may not be certain whether the combination of their specific ingredients

would yield the same result. Consequently, further studies on the active ingredients of the two plant materials possessing anti-*Salmonella typhi* property in this study is hereby recommended. This is imperative especially in recent times when typhoid fever ranks high as one of the most common ailments among all age groups in the less developed nations.

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