

An evaluation of the antimicrobial synergy of Garlic (*Allium sativum*) and Utazi (*Gongronema latifolium*) on *Escherichia coli* and *Staphylococcus aureus*

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ABSTRACT

As part of the on-going search for potent and resistance-free antimicrobial medicinal plants, the antimicrobial and synergistic effects of the plants, *Allium sativum* (E1) and *Gongronema latifolium* (E2) on *Escherichia coli* and *Staphylococcus aureus* were investigated. The sensitivities of *E. coli* and *S. aureus* to E1 and E2 and the minimum inhibitory concentrations of the plant extracts, individually and in combination with themselves, and with ciprofloxacin (CPX) and ampicillin (AMP), were tested using standard procedures. E1 and E2 individually showed appreciable antimicrobial effect (zones of inhibition ≥ 16 mm). The combination of E1 and E2 against the test organisms was not effective due to antagonism between E1 and E2. E1 or E2 when combined with CPX, completely suppressed the effect of CPX against *E. coli*, and rather produced additive effect on *S. aureus* similar to the combination of E2 and AMP against *S. aureus*, although CPX alone was more effective than either E1 or E2, unlike AMP. Synergism was observed in the combination of E1 and AMP against *S. aureus*. It is concluded that synergism associated with the combination of medicinal plants is doubtful. However, the synergistic or additive effect between garlic and conventional drugs to some strains of bacteria which are resistant to some conventional drugs, gives hope of fighting drug resistance.

Keywords: antimicrobial effect, synergism, *Allium sativum*, *Gongronema latifolium*, ciprofloxacin, ampicillin

INTRODUCTION

Garlic (*Allium sativum*) in the family Liliaceae is a perennial bulb-forming plant. It is known world-wide, and for several centuries, it has been used for dietary and medicinal purposes (Ross *et al.*, 2001). *Gongronema latifolium*, of the family Asclepiadaceae (locally called utazi in the Igbo dialect of Nigeria), is a climber with woody hollow glabrous stems below, and characterized by greenish yellow flowers. The leaves of the plant are used as spice and as medicine in West Africa.

Several studies have confirmed that garlic has antimicrobial properties (Reuter *et al.*, 1996; Martin and Ernst, 2003; Eja *et al.*, 2007). On the other hand, not much work has been carried out to investigate the antimicrobial activity of *G. latifolium*. One such successful research, among a few others, was reported by Oshodi *et al.* (2004) on the antimicrobial activity of aqueous extracts of *Vernonia amygdalina*, *Garcinia kola*, and *G. latifolium* and their blends, on some beer spoilage organisms. The application of blends was based on the concept of the effectiveness of combination therapy (synergism) in the treatment of certain bacterial infections involving drug resistant organisms (Weinstein, 1975; Mitchel *et al.*, 1977; Duncan *et al.*, 1980). Garlic has even been noted to act synergistically with antibiotics (Sivam *et al.*, 1997); and no similar specific report has been made on *G. latifolium*. Recently, however, *Lasianthera africana* or *Heinsia crinata* in combination with chloramphenicol, has been

observed to have synergistic effect on *Candida albicans* (Andy *et al.*, 2008).

However, synergic treatment of ailments has been a long aged practice in both orthodox and traditional medicines. In Sub-saharan Africa, especially West Africa, including Ghana and Nigeria, herbal medicines are believed to be more effective when taken in combination. In particular, herbal medicines taken orally are combined and immersed in locally brewed gin for a short period of time for effective extraction of the active ingredients of the herbs. The gin extracts of the herbs are taken in homes, or sold in wine bars as medicines against some ailments.

The aim in this study was to assess the antimicrobial activity of garlic extract compared to that of the less widely known *G. latifolium* in parallel with ciprofloxacin and ampicillin as control, against known bacterial pathogens such as *Escherichia coli* and *Staphylococcus aureus*. The antimicrobial activity of the extracts against the organisms was also to be tested in combination with themselves and with the controls (ciprofloxacin and ampicillin).

MATERIALS AND METHODS

Source of test organisms and plants

Known cultures of *E. coli* and *S. aureus* were obtained from the Department of Medical Microbiology and Parasitology of the University Teaching Hospital, Calabar. The two test plants, garlic and utazi, were bought directly

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from women who are the main sellers of spices at Calabar Central Market.

Preparation and concentrations of the garlic and utazi extracts

The garlic cloves were peeled, cut into pieces, and 20 g was put in a juice extractor to squeeze out the raw garlic extract. Utazi was pounded in a clean mortar and the raw extract was squeezed out.

Each of the extracts (10.5 mL) was placed in a pre-weighed glass dish and reweighed, followed by drying in an oven at 60 °C. The difference between the weights of each of the extracts before and after drying, was taken as the weight of the active component of the extracts which could have evaporated. The weights obtained for garlic (Eja *et al.*, 2007) and utazi extracts were 1.12 mg and 1.21 mg respectively, equivalent to 106.7 and 115.2 mg/mL.

Thus, from the raw garlic and utazi extracts, dilutions of 1:2, 1:4, 1:8, 1:16, 1:32 and 1:64 in deionised water, corresponding to 35.6, 21.3, 11.8, 6.3, 3.2, 1.6 mg/mL for garlic, were made, while the corresponding weights for utazi were 38.2, 23.0, 12.8, 6.8, 3.5 and 1.8 mg/mL. These were calculated based on the ratios. For instance, 1:2 resulted in $\frac{1}{2} \times 106.7$ mg/mL, and so on to 1:64 which gave $\frac{1}{64} \times 106.7 = 1.6$ mg/mL. These dilutions were used for the minimum inhibitory concentration (MIC) test.

Testing for antimicrobial effects of raw garlic and utazi extracts

A disc diffusion technique using the Kirby-Bauer method (Prescott *et al.*, 2005) was applied in testing pure cultures of the isolates for their antimicrobial sensitivities.

The sensitivity discs used for the test were punched from Whatman No. 1 filter paper. The discs were 5 mm in diameter. After sterilization, the discs were impregnated with the raw extracts of garlic and utazi separately (Onyeaba *et al.*, 2004). Five agar plates for each test organism per plant extract were inoculated with 0.1 mL broth culture of test organisms and spread with a glass rod shaped like a hockey stick, and incubated at 37 °C for 24 h. The antibiotics, ampicillin (AMP) and ciprofloxacin (CPX), were used as controls for comparison with the raw extracts. The plates were observed for zones of inhibition after incubation followed by calculation of the mean zones of inhibition (mm).

Testing for MIC of the extracts

The MIC was determined by first preparing a standard inoculum. This involved transferring a portion of the pure culture of each isolate into tryptone soya broth (Oxoid CM129) and incubating at room temperature overnight. The overnight broth culture (0.1 mL) was diluted with 1 mL of distilled water in the ratio of 1:100 to give a final dilution of 10^{-3} of the standard inoculum (Adoum *et al.*, 1997) following which the dilution susceptibility test technique (Prescott *et al.*, 2005) was applied. The reciprocal of 10^{-3}

was the number of organisms in the standard inoculum used for the MIC test. In this technique, the previously prepared Mueller-Hinton broth containing various concentrations of the extracts of the plants was inoculated with the standard inoculum. This was done for each of the test organisms followed by incubation at 37 °C for 16–20 h. The presence or absence of growth for each concentration was recorded at the end of incubation. The lowest concentration of the extracts resulting in no growth after 16–20 h of incubation was taken as the MIC. The same treatment was given to the combination of the extracts of the two plants and those of the individual extracts.

Synergy test

To test the combined extracts, equal volumes (0.1:0.1) were mixed and the mixture was tested with the individual extracts separately.

To carry out a combination test of the extracts with the controls (CPX and AMP), 106.7 and 115.2 mg of CPX and AMP respectively, were each dissolved in 1 mL of deionized water, so that the initial concentrations of the controls were the same as those of the raw extracts. Thus, to test the extracts combined with CPX and AMP, equal volumes of the extracts and the controls (0.1:0.1) were mixed and the mixture was tested along with the individual extracts and controls separately (Andy *et al.*, 2008).

RESULTS

Table 1 represents the effect of the extracts of *A. sativum* (E1) and *G. latifolium* (E2) on *E. coli* and *S. aureus*. The zones of inhibition produced by E1 and E2 were more than 15 mm indicating appreciable antimicrobial activity against the test organisms. In the combination of E1 and E2, the effect of E2 on *E. coli* was completely antagonized or masked by that of E1. With respect to *S. aureus*, there was antagonism between E1 and E2 resulting in a lower combination effect (13 mm zone of inhibition) than either E1 or E2. E2 antagonized E1 but not completely. The table shows that CPX was more effective than E1 or E2 against both *E. coli* and *S. aureus*. AMP was rather less effective than the plants, and *S. aureus* was especially resistant to AMP.

The effect of the combination of the plant extracts with conventional antibiotics, CPX and AMP is represented in Tables 2 and 3. CPX was completely antagonized by E1 and E2 in their combined effect on *E. coli*. On the other hand, the combination of CPX with E1 produced additive effect on *S. aureus*, while E2 partially antagonized CPX (Table 2).

However, the combination of AMP with E1 showed additive effect on *E. coli* and synergistic effect on *S. aureus* as shown in Table 3. On the effect of the combination E2 + AMP against the test organisms, there was additive effect between E2 and AMP on *E. coli* (Table 3) and antagonism between AMP and E2 on *S. aureus*. The combination of the MIC of E1 and E2 on the test

Table 1: Effect of raw extracts of *A. sativum* (E1) and *G. latifolium* (E2) on the test organisms

Test organisms	Mean zones of inhibition (mm)				
	E1	E2	E1 + E2	CPX	AMP
<i>Escherichia coli</i>	19 ^a	17	19	30	13
<i>Staphylococcus aureus</i>	18	16 ^b	13	38	0*

* *Staphylococcus aureus* was resistant to ampicillin.

^a E1 completely antagonized E2; ^b = E2 antagonized E1 resulting in a lower figure of 13 (E1 + E2).

Table 2: Effect of raw extracts of *A. sativum* (E1), *G. latifolium* (E2) and ciprofloxacin (CPX) on the test organisms

Test organisms	Mean zones of inhibition (mm)				
	E1	E2	CPX	E1 + CPX	E2 + CPX
<i>Escherichia coli</i>	19 ^a	17 ^b	30	19	17
<i>Staphylococcus aureus</i>	18	16	38	42	31

^a E1 completely antagonized CPX to result in 19 (E1 + CPX).

^b E2 completely antagonized CPX to result in 17 (E2 + CPX).

Table 3: Effect of raw extracts of *A. sativum* (E1), *G. latifolium* (E2) and ampicillin (AMP) on the test organisms

Test organisms	Mean zones of inhibition (mm)				
	E1	E2	AMP	E1 + AMP	E2 + AMP
<i>Escherichia coli</i>	19	17	13	21	18
<i>Staphylococcus aureus</i>	18	16 ^c	0*	23	10

* *Staphylococcus aureus* was resistant to ampicillin.

^c E2 was antagonized by AMP.

Table 4: The minimum inhibitory concentration (MIC) of *A. sativum* (E1) and *G. latifolium* (E2) singly and in combination on the test organisms

Test organisms	MIC (mg/mL)		
	E1	E2	E1 + E2
<i>Escherichia coli</i>	3.2	6.3	3.2
<i>Staphylococcus aureus</i>	11.8	21.3	15.6

Figures represent means of two readings obtained from the dilutions of E1 (106.7 mg/mL) and E2 (115.2 mg/mL) from 1:2 to 1:64.

organisms produced antagonistic effect on both *E. coli* and *S. aureus* (Table 4). E1 completely masked the effect of E2 against *E. coli*, but partially antagonized E2 in the case of *S. aureus*.

DISCUSSION

Results obtained in this study have revealed that *A. sativum* and *G. latifolium* have very high antimicrobial activity (mean zone of inhibition ≥ 16 mm) against the test organisms, *E. coli* representing the group of gram negative enteric bacterial pathogens, and *S. aureus* representing the group of gram positive bacterial pathogens. Of course, it has been reported and established that *A. sativum* has antimicrobial potency (Cai *et al.*, 2007; Eja *et al.*, 2007). There are reports on the antimicrobial activity of *A. sativum* against diarrhoeagenic pathogens, e.g., *E. coli*, and wound pathogens, such as *S. aureus* (Tessema *et al.*, 2006; Eja *et al.*, 2007). The antimicrobial activity of the garlic has been attributed to its phytochemical component, allicin (a thiosulfonate) whose removal completely renders garlic ineffective against microorganisms (Hughes and Lawson, 1991; Cai *et al.*, 2007).

However, not much research has been carried out on the antimicrobial property of *G. latifolium* which has been used for ages by the people of West Africa including Nigeria, for dietary and medicinal purposes. The antimicrobial activity revealed in this study had been reported in an earlier study (Oshodi *et al.*, 2004). The phytochemical composition of *G. latifolium* has been reported to consist of about 0.5% flavonoids, 2% tannins, 0.66% saponins, 0.33% polyphenols, 1.97% alkaloids and 13.2% hydrogen cyanide (Atangwho *et al.*, 2009). The presence of tannins, alkaloids, flavonoids, saponins and polyphenols could have been responsible for the antibacterial activity of *G. latifolium*. Elsewhere it is reported that tannins, alkaloids, saponins, flavonoids and glycosides could be associated with the antimicrobial activities of some Nigerian plants (Andy *et al.*, 2008; Ibrahim *et al.*, 2009). Of course, Farombi (2003) has noted that the active components of some of the Nigerian medicinal plants reside in the phytochemical components mentioned above. What has not been resolved is the separation of the specific bioactive components against specific organisms; and this has been noted to affect the quality and safety of herbal medicines (Sane, 2002).

In the combination of the extracts of *A. sativum* (E1) and *G. latifolium* (E2), it is apparent that there is greater effectivity using *A. sativum* alone for the treatment of infections associated with the two test organisms. A similar pattern of effect was observed in the combination of the extracts of the test plants with CPX and AMP. It is not known why CPX alone was more effective than garlic in this study, whereas it is the opposite in an earlier study (Eja *et al.*, 2007). This could have been attributed to the differences in the concentrations of garlic and CPX used in the two separate studies.

This study reveals that the combination of garlic and utazi may not always yield the desired effect on both

Gram positive and Gram negative bacteria, as there is evidence of antagonism among the plants, even in combination with conventional drugs. There is an indication therefore, that the antimicrobial effect of very highly active chemotherapeutic drug could be suppressed or masked when taken together with some medicinal plants. This agrees with the findings of Andy *et al.* (2008). The antagonistic effect is more evident when the combination is used on Gram negative organisms, e.g. *E. coli*. There is promise of desired effect when the combinations are used on Gram positive bacteria, e.g. *S. aureus*, in which case, the effect may be additive or synergistic. Additive effect as applied in this study means an enhanced effect produced by the combination of two or more plant extracts, or extracts combined with conventional drugs. The effect is antagonistic when one plant suppresses the effect of the other resulting in a diminished effect, and synergistic when the elevated effect potentially destroys a resistant organism, as experienced in the effect of the combination of garlic and AMP against *S. aureus* in this study.

Similar findings are available (Andy *et al.*, 2008). However there were conflicting reports on the combined effects of *V. amygdalina*, *G. kola* and *G. latifolium* on some microbial species, including *E. coli* and *S. aureus* in a study of the antimicrobial activity of Nigerian medicinal plants potentially usable as hop substitute (Oshodi *et al.*, 2004). In the report (Oshodi *et al.*, 2004), *G. latifolium* appeared to have little or no effect on *E. coli*, but the combination of *G. latifolium*, *G. kola* and *V. amygdalina* had high effect on *E. coli*, *S. aureus* and *Streptococcus salivarius*. Apparently, high concentrations were used besides combining three plant extracts. Even then, the report of Oshodi *et al.* (2004) did not deviate much from the findings of this study.

CONCLUSIONS

It is concluded that *A. sativum* and *G. latifolium* individually possess relatively high antimicrobial activity against *E. coli* and *S. aureus*. However, the combined effects of the plant extracts on these organisms are in doubt especially as the factors of antagonism and/or additive effects are not yet properly elucidated. What is apparent is the synergism between AMP and garlic on *S. aureus* which was noted to be completely resistant to AMP. The synergism observed in this study is attractive in view of the current problem of drug resistance by microorganisms and the fact that the modes of action of garlic and ampicillin (i.e. inhibition of cell wall synthesis) are similar (Prescott *et al.*, 2005; Eja *et al.*, 2007).

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