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The Response of *Staphylococcus aureus, Klebsiella pneumoniae* and *Streptococcus pneumoniae* to Plant Derived Nutraceuticals

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Abstract **Objective**: The present study evaluated the antimicrobial properties of selected plant derived nutraceuticals against S*taphylococcus aureus, Klebsiella pneumoniae* and *Streptococcus pneumoniae*. **Material and Method**: The plants utilized were *Zingiber officinal*e, *Aloe barbadensis miller, Alipina galanga,* A*llium sativum,* three varieties of *Allium cepa,* and *Moringa oleifera*. S*taphylococcus aureus, Klebsiella pneumoniae* and *Streptococcus pneumoniae* were the microorganisms employed in the study due to the frequency with which they are implicated in several infections and diseases such as boils, skin infections, respiratory diseases and food poisoning. Standard microbiological methods were employed. The microorganisms were collected and identified by a microbiologist and the plant samples were identified by an agronomist. The microoganisms were sub-cultured and the disc diffusion and turbidity testing techniques were applied. Combination tests were done with each herb aimed at ascertaining the efficacy of the herbs. **Results:** The findings demonstrated that garlic in all its concentrations, with both methods (disc diffusion and turbidity) and in all combinations produced remarkable zones of inhibitions against all microorganisms (*Staphylococcus aureus, Klebsiella pneumoniae* and *Streptococcus pneumoniae*). Ginger showed no zone of inhibition regardless of combinations, except when combined with garlic. The variety of onions used showed intermediate zones while *Alipina galanga* and *Moringa oleifera* showed no visible antimicrobial properties. **Conclusion**: Garlic and the variety of onions used, in all dilutions were seen to possess antimicrobial properties against *Staphylococcus aureus, Klebsiella pneumoniae and Streptococcus pneumonia*e when compared to the conventional antibiotics used.

Keywords: *resistant, plants derived nutraceutical, pathogenic bacteria, antimicrobial properties*

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1. Introduction/Literature Review

This research seeks to investigate the scientific proof of the efficacies of some regular herbs used in topical and systemic treatment of some illnesses locally in Jamaica. A total of six communities where visited in both parishes for the collection of the *Aloe vera* plants.

The parishes of Manchester and Clarendon are widely known for their growth of *Aloe vera* plants and ginger roots as botanical plant in many gardens. The antioxidant capacity of common fruits and vegetables has been analyzed in some studies [1]. These studies demonstrated a wide diversity of antioxidant capacities among the common fruits and vegetables. The antioxidant capacity can largely be attributed to the phenolic compounds, such as phenolic acids, flavonoids, and anthocyanins, found in plants [2]. It is evident that the importance of the use of these herbs in the study was vital. The current national and annual consumption of onion is roughly 7.4 million kilograms of which 500,000.00 kilograms or seven percent 7% are locally produced [3]. It is believed that onions have phyto-chemicals that may improve the working of vitamin C in the body, thus aiding with improved immunity. A powerful compound called quercetin in onions is known to play a significant role in preventing cancer [4]. Onions may even be used on some insect bites such as that of the honey bee where immediate relief from pain is said to occur [4].

The increased resistance of persons to antimicrobial therapy has become a matter of grave concern in recent times. This increase may be due to the frequency with which antibiotics are presently administered to patient coupled with ill compliance on the part of the patients. Some infections are communicable, and are therefore, hard to control. It is with this understanding that we decided to look deeper into the notion, that routinely used herbs do possess antimicrobial properties, (with little or no clinically significant side effects) and eventually can be used as adjunctive treatment to some conventional antimicrobial therapies.

A study of this nature was important to be conducted as the derived plant nutraceutical has not yet been utilized in studies to ascertain whether these locally utilized herbs possess microbial properties under these conditions

against the bacteria in question. This we hope will result in increased treatment choices of natural origin which may even result in quicker recovery for patients having resistance to antibiotic therapy. These natural treatments are projected to decrease morbidity, mortality and the risk of antibiotic resistance which often occurs when medical practitioners administer antibiotics but are unaware of the patient's resistance. The spread of antimicrobial resistance is one of the most important emerging infectious disease threats in developed and developing countries. The choice of microorganisms used, came as a result of the frequency with which illness from these pathogens are reported and the natural herbs that are commonly used in an attempt to suppress or eradicate these causative agents. Herbs have been used for years to assist in the recovery and prevention of many illnesses but little is known about their true effectiveness in their natural state against bacterial infections. Recent studies show that the emergence of resistant strains to antibiotic is a cause for concern. The goal of this research is to better understand the antimicrobial properties, if any to the herbs selected against *S. aureus, K. pneumoniae* and *Streptococcus pneumoniae*. The results from this study will aid in the prescription of herbs to be used in therapeutic medicine having little or no side effects, unlike other therapeutic drugs.

2. Material and Method

Microorganisms.

Three different characterized drug resistant bacterial strains including: S. aureus, S. pneumoniae, and K. pneumoniae were utilized. The strains were subcultured and maintained on blood agar plates at 37°C.

Plants.

The plants utilized were *Zingiber officinal*e, *Aloe barbadensis miller, Alipina galanga, Allium sativum,* three varieties of *Allium cepa*, Yellow-White-Purple Onion (*Allium cepa*) and *Moringa oleifera*.

Table 1. concentration of Plant materials

Nutraceutical plant	Codes	Concentration
Galangal Leaves	GG1-Unquailed,	100%
	GG2- Unquailed	100%
	GG3-Quailed,	100%
	GG4- Quailed	100%
Garlic	GC1-1 clove	25%
	GC2-2cloves	75%
	GC3-3 cloves	100%
Aloe Vera- Manchester	M1	100%
	M2	100%
	M3	100%
	M4	100%
	M5	100%
	M6	100%
Aloe Vera-Clarendon	C1	100%
	C2	100%
	C3	100%
	C4	100%
	C5	100%
	C6	100%
Ginger	OG-A-Old ginger harvested in Manchester	100%
	OG-B- Old ginger harvested in Clarendon	100%
	FG-A-Fresh Ginger harvested from Manchester	100%
	FG-B- Fresh ginger harvested from Clarendon	100%
Onion	YO	100%
	WO	100%
	PO	100%

2.1. Procedure for the Disc Diffusion Technique of Selected Plants Derived Nutraceutical

All selected plants from Manchester and Clarendon were collected.

The plants were washed three times with potable and distilled water respectively and dried.

The bulb (*Allium sativum, Allium cepa*), leaves (*Aloe barbadensis miller, Alipina galangal*), rhizome (*Zingiber officinal*e), seed (*Moringa oleifera)* plant derived nutraceuticals were blended in 20mL of sterile distilled water and then filtered using Whatman No. 1 filter paper after which dilutions of the concentrate {(1:1-50 ul of a queous plant extract) 1:2 - 25ul of aqueous plant extract mixed with 25ul of sterile distilled water, 1:4-15 ul of aqueous plant extract mixed with 35ul of sterile distilled water)} were made. The entire bulb, root, gel from the leaf, peg was used due to the fact that the study looked at the plant in its most natural state. The pre made discs were impregnated with the extracts and were allowed to soak at room temperature for 1hr. A colony suspension of each micro-organism to be tested was prepared to a 0.5

McFarlane Standard. The continuous streaking technique was used. Heat sterilized forceps were used to remove the sample soaked discs and placed on the streaked Mueller Hinton agar plates along with combination testing. Antibacterial activities were calculated as a mean of 3 replicates. Diameter of the clear zones, showing no bacterial growth, around each disc was measured with the help of vernier caliper. Triplicate plates were prepared for each sample.

The plates were incubated for 24hrs at 37°C prior to interpretation of antimicrobial susceptibility test results.

2.1.1. Procedure for the Turbidity Technique Plant Derived Nutraceuticals

The plant derived nutraceuticals that were diluted in different concentrations were then transferred in four (4) mL sterile tubes, an estimate of 2 mL of the diluted plant derived nutraceutical were placed into the test tubes. A sterile stab was then used to inoculate the test organisms into the turbidity tubes.

The inoculated tubes were incubated for 24hrs at 37°C prior to interpretation of turbidity test result and compared with the antimicrobial susceptibility test results.

2.2. Statistical Analysis

Discrete data corresponding with the various zones of inhibition were represented by the use of the IBM SPSS version 22. The chi-square test was used to compare the zone inhibition utilized from the nutraceutical disc used and the conventional antibiotic used. Student T-test was then used to determine the difference between the mean zone of inhibition of the plants and the conventional drugs.

3. Result

The results showed that not all herbs possessed antimicrobial properties against microorganisms tested. The combined effects showed slight variation with some zonal size for *S. aureus*, *K. pneumoniae* and *Streptococcus pneumoniae*. The organisms were pretested using commercial antibiotics such as chloramphenicol for comparative analysis.

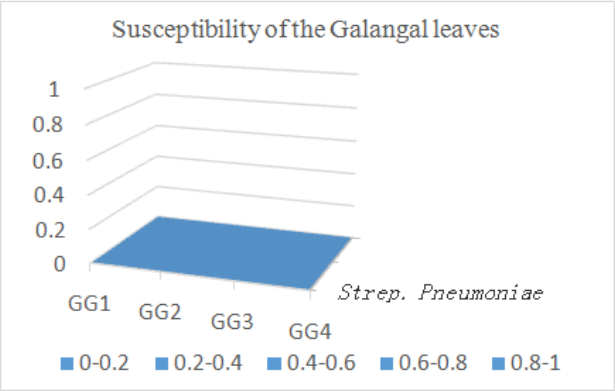

Figure 1. X-axis GG1-Unquailed, GG2- Unquailed, GG3-Quailed, GG4-Quailed Y-axis concentration of galangal leaves

The results indicated a zone of 19 mm against *S. aureus*, 21mm against *Streptococcus pneumoniae* and 19 mm against *K. pneumoniae*. Penicillin produced zones of 30 mm against *S. aureus*, 24 mm against *Streptococcus pneumoniae* and 16mm against *K. pneumoniae*. Gentamycin showed 15 mm, right across the board for all organisms and Tetracycline produced 19mm for both *S. aureus* and *Klebsiella pneumonia* and for *Streptococcus pneumoniae* showed a zone of 23 mm. These were used as additional control measures. Regardless of the methods of treatment used for the *Alipina galangal* leaves, Ginger, *Aloe Vera* and *Moringa oleifera* no visible antimicrobial properties were seen. (Figure 1).

Garlic in all its strengths produced zonal levels of inhibition as seen in Figure 2. The one clove of garlic preparation (GC1) was used against *S. aureus* and produced a zone of inhibition of 7mm. The herbal preparation used against *K. pneumoniae* and *Streptococcus Pneumoniae* produced a zone of Inhibition of 8mm and 7mm respectively. As the concentration of garlic increased so did the overall zonal measurements. The reactivity of 2 cloves of Garlic GC2 on *S. aureus* measures 15 mm. *K. pneumoniae and Streptococcus pneumoniae* all produced the same zone of 15 mm. The garlic preparation containing 3 cloves, produced even larger zone of Inhibition for the organisms used, ranging from 20 mm to 25 mm. The Largest noted reactivity was observed with *Staphylococcus aureus*, this produced a zone of 25 mm. With the herbal treatment for *Klebsiella pneumoniae* the zone of inhibition 24 mm was observed. *Streptococcus pneumoniae* showed a zone of inhibition of 20 mm. *S. aureus* when tested against yellow (YO), white (WO) and purple (PO) onion had zones of inhibition of 15 mm, 10 mm and 10 mm respectively. *K. pneumoniae* when tested against yellow (YO), white (WO) and purple (PO) onion produced zones of inhibitions of 10 mm for all herbs used. When *Streptococcus Pneumoniae* was tested against the herbs, all also produced the same sized zone of inhibition of 10 mm respectively.

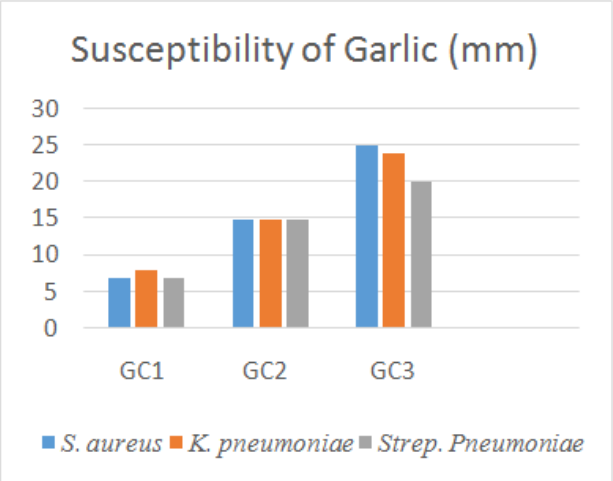

Figure 2. GC1-25% strength (1 clove), GC2-50% strength (2 cloves), and GC3-75% strength (3 cloves)

3.1. Combination Testing

Garlic when combined with Ginger produced zones of inhibition of 5mm for all microorganisms. Garlic GC1, when combined with white onion, a zone of 10mm was observed *for S. aureus*, 7 mm for *Streptococcus pneumoniae* and 7 mm for *K. pneumoniae*. The combination of garlic with purple onion showed zones such as 10 mm, 10 mm, and 7 mm for *S. aureus*,

Streptococcus pneumoniae and *K. pneumoniae* respectively. With the application of (Garlic) GC1 and (Yellow onion) YO the zone of inhibition was measured as 15 mm for *S. aureus, while K. pneumoniae* and *Streptococcus pneumoniae* measured 10 mm respectively. When compared to the herbs in their uncombined state it was noted that the zone of inhibition for *S. aureus* decreased when White onion was combined with GC1 from 13 mm to 7 mm. Garlic (GC1) in its smallest concentration produced a zone of 7 mm when treated against *S. aureus*. GC1 when combined with YO against *S. aureus* produced a zone of 15 mm. However the combined effect resulted in zone sizes of 10mm for *S. aureus* and 7 mm for *Streptococcus pneumoniae. Streptococcus pneumoniae* showed decreased zone sizes with a difference of 3 mm. Turbidity testing were done in order to confirm the findings Figure 3.

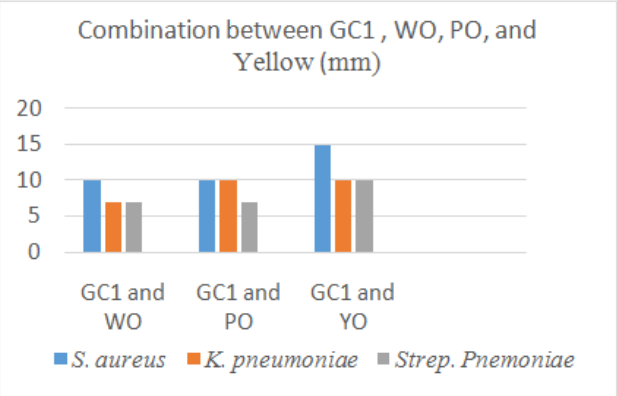

Figure 3. Combination of garlic 25% strength with white onion, purple onion and yellow onion

4. Discussion

The spread of antimicrobial resistance is one of the most important emerging infectious disease threats in developed and developing country [4]. The present study was undertaken to evaluate the antimicrobial properties of specific herbs against *S. aureus, K. pneumoniae* and *Streptococcus pneumoniae*. Herbs contain healing components which help the body to absorb and utilize nutrients while safeguarding against side effects [5]. The herbs in their natural form were used instead of extracted potions due to the fact that ordinarily, the greater population of the Jamaican people would have access to the whole plant against extracted constituents. There is also an advantage to using the whole herb or whole herbal products instead of a single extracted constituent [5]. It was found that some herbs from the rhizome family such as ginger, turmeric and *Alipina galanga* possess properties such as being a diaphoreritic, digestive, parasitical, and stimulant etc; [5]. The results indicated that ginger and *Alipina galanga* does not possess antimicrobial properties against *Staphylococcus aureus, Klebsiella pneumoniae* and *Streptococcus pneumoniae*. This was evident due to the lack of zone of inhibition seen and the increase turbidity observed after repeated studies over a 24 hours period. This may have been as a result of the use of only aqueous extraction methods against other non-aqueous methods which if used may have increased the extraction properties to increase the viability of antimicrobial agent if present.

Garlic (*Allium sativum*), in all its strengths produced remarkable zonal level of inhibition. Garlic has been used for food and medicine since the time of ancient Egyptians. It was found to be equal in activities to penicillin, but without the harmful side effects [5]. The one clove of garlic preparation (GC1) tested against *S. aureus* produced a zone of inhibition of 7 mm. The herbal preparation tested against *K. pneumoniae* produced a zone of inhibition of 8 mm, *Streptococcus pneumoniae* had a zone of 7 mm. The two clove preparation (GC2) were used against *S. aureus, K. pneumoniae* and *Streptococcus pneumoniae* all produced the same zone of 15 mm. The garlic preparation containing 3 cloves, produced even larger zone of inhibitions for the organisms tested, ranging from 20 mm to 25 mm. When GC3 was compared to conventional Tetracycline using chi square, X^2 cal (7.265) >2.021 at df 40. 95% confidence was therefore received. This suggests that there is a significant difference in antimicrobial activity between selected herbs when compared to conventional drugs. According to Lemar et al. [6] in determining whether *Allium sativum* (garlic) extract has any effect on the morphological transformation of *Candida albicans*, and they investigated whether it could alter the gene expression level of SIR2, a morphogenetic control gene and SAP4, a gene encoding secreted aspartyl proteinase. The results revealed that the hyphal production was an essential virulence determinant of *C. albicans* for invasive infections, therefore garlic and its constituents can be effective not only against colonizing *C. albicans* strains present in mucosal infections, but also virulent strains causing systemic or invasive candidiasis. Some researchers indicaticated that, "The efficacy of Aloe liquid in combination with selected antibiotics as an antibacterial agent showed a wide range against Gram positive and Gram negative bacteria" [7,8,9,10]. This was however slightly different when compared to this present study as the assay methods used were vastly different along with the conventional combinational approach.

Moringa oleifera, a plant deemed to be a cure all, showed no zone of inhibition. *Staphylococcus aureus* when tested against yellow, white and purple onion had zones of inhibition of 15 mm, 10 mm and 10 mm respectively. *Moringa oleifera* leaf was tested by [11] who found that the properties in the leaf had antimicrobial strength. *K. pneumoniae* and *Streptococcus pneumoniae* when tested against yellow, white and purple onion produced zones of inhibitions of 10 mm respectively. The results observed for onion agrees with the observations of [12] who reported moderate antibacterial activity of onion extract against *E. coli, S. paratyphi* and *S. typhimurium*. [13] They reported that there was good antibacterial activity of onion extract on the growth of *S. enteritidis*.

Alipina galanga when combined with garlic produced a zone of 5 mm for all bacteria tested. Garlic when combined with ginger also produced zones of Inhibition of 5 mm for all organisms. When garlic (GC1) was combined with white onion, a zone of 10 mm was observed for *S. aureus*, 7 mm for *Streptococcus pneumoniae* and 7 mm for *K. pneumoniae*. The combination of garlic with purple onion showed zones such as 10 mm, 10 mm, and 7 mm for *S. aureus, Streptococcus pneumoniae* and *K.*

pneumoniae respectively. The antibacterial activity of garlic is reported to be due to the action of allicin or diallyl thiosulphinic acid or diallyl disulphide

5. Summary and Conclusion

The goal of this research was achieved as Garlic and the variety of onions used showed remarkable antimicrobial properties measured by the use of zones of inhibition. The potential therapeutic benefits of herbal medicine has for years been on the spot light of emerging studies. This study focused on three clinically significant bacteria namely *Staphylococcus aureus, Klebsiella pneumoniae* and *Streptococcus pneumoniae* due to their occurrence in bacterial illnesses and their reported frequency with resistance therapy. The antimicrobial properties of Ginger, Aloe Vera, *Moringa oleifera*, Garlic, *Alipina galanga*, and the three varieties of onion in their natural states were the central focus as they were used against the above listed bacteria. Garlic, yellow, white and purple onion all showed large zones of Inhibition when compared to regularly used antibiotics. The comparison was vital as this became the basis for measuring the validity of the zones of inhibition.

The scope of the study allowed for the use of the disc diffusion technique in zonal measurements, compared with the turbidity test.

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