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Antibacterial and Synergistic Effects of Extracts of *Allium cepa*, *Allium sativum*, *Zingiber officinale* and *Garcinia kola* on Selected Bacterial Strains

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ABSTRACT

The rising cases of antibiotic resistance and the failure of many drugs has led to growing calls for a return to nature and the fighting powers of many natural products against infections as solutions. Therefore, this study was aimed at investigating the phytochemical, antibacterial and synergistic potency of the extracts of some of these natural plants viz.: *Allium cepa, Allium sativum, Zingiber officinale* and *Garcinia kola* against some selected bacterial strains which are sometimes used in antimicrobial resistance studies: *Listeria monocytogenes, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Qualitative phytochemical analysis of the extracts showed that they contain varying amounts of alkaloids, flavonoids, glycosides, tannins, saponins and steroids. Aqueous, ethanol and hexane extracts of the plants were used in the investigations against the test microorganisms. Results obtained showed that the microorganisms were highly susceptible to the aqueous and ethanol extracts, but showed comparatively less susceptibility to hexane extracts which were mainly oily components. Compared to the other test strains, *K. pneumoniae* showed the most resistance to the extracts. Combinations of the extracts showed positive synergism as they produced higher antimicrobial effects on many of the organisms. The minimum inhibitory concentration (MIC) ranged from 25mg/mL to 200mg/mL. Aqueous extracts of the plants produced the lowest MIC value of 25mg/mL against most of the bacteria. Considering the increasing failure of conventional antibiotic therapies, the results of this study could be positive indications that natural plants, spices and foods could serve as possible alternatives or options in the management of many infections.

Keywords: Antimicrobials, Medicinal plants, Minimum Inhibitory Concentration (MIC), Phytochemicals, Synergism.

Introduction

One of the prime pursuits of science today is the search for alternatives to our failing antibiotics. This is because the unusually high rate of antibiotics resistance currently prevalent all over the world, has become one of the worst challenges of our time. The search for good alternatives is therefore now an urgent existential need.[1] The World Health Organization put it aptly when it said that the increasing rate of antibiotic resisting bacteria threatens the achievements of modern medicine.[2] Pointing out that it can affect anyone irrespective of age or country, the organization highlights its threat not just to global health, but also food security and general development today.[3] The present time is considered an era where microorganisms develop resistance faster than new antibiotics are develop.[3]
The need for an intensive and extensive search for new and more effective antimicrobial agents is therefore urgent and necessary. One way of achieving that goal is through the time-worn advice attributed to Hippocrates: "Let food be thy medicine and medicine be thy food". That advice has however come under increasing criticism by many as being akin to 'veneration of food as medicine' used to convince skeptics to accept the outlandish claims by many medicinal food enthusiasts.[5] However, it may not be true that such claims are outlandish after all. Nutritionists and many researchers know that food is truly not just always peculiarly concerned with quenching our hunger pangs; a lot of its contents such as spices are known to have many medicinal and interestingly positive health attributes many of which are yet undiscovered.[6,7] Therefore, the need for research to situate the veracity or otherwise of many of the claims cannot be over-emphasized.
Among the many spices and food condiments used all over the world, only very few enjoy the global reach and acceptance as onions, garlic and ginger as their use is truly widespread. Onions and garlic belong to the same family of plants Alliaceae, hence, their botanical names, *Allium cepa* for onions and *Allium sativum* for garlic.[8] They are both known for their sharp tastes and recognizable odors which easily give them away. They also have faint similarities in appearance as both are rounded bulbs. They have been used as medicines in many ancient cultures dating back to thousands of years. Ginger on the other hand belong to the family Zingiberaceae, hence its name *Zingiber officinale*.[9] It is a flowering plant with leafy stems and greenish yellow flowers, but it is the root, preferably called rhizome that is the source of its spices and medicinal qualities. Deceptively called African ginger sometimes, it is considered native to the warmer parts of Asia China, India and Japan, but is grown all over the world today including in the Middle East, Africa and South America.[10] Finally, *Garcinia kola* otherwise called bitter kola is found in the Southern part of Nigeria and a few other African countries like Democratic Republic of Congo and often referred to as 'wonder plant because every part of it is believed to have medicinal importance.[11,12]

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The need to continually situate the importance of food and many of its ingredients including commonly used spices and nuts as potent medicinal substances in addition to their nutritional and hunger assuaging roles necessitates extensive, diverse and reiterative investigations. These will help provide more lasting solutions to the problem of antimicrobial resistance. Bearing these in mind, this study was aimed at investigating the antibacterial activity of these important and truly strategic plants as additional proofs of food as medicine. To do so three extracts of each of them: water (aqueous), ethanol and hexane were used against some selected bacterial strains.

Materials and Methods

Sample collection
Fresh samples of onions (*Allium cepa*), garlic (*Allium sativum*), ginger (*Zingiber officinale*) and bitter kola (*Garcinia kola*) used in the study were bought from the local Ogige market, located along University Market Road in Nsukka, Enugu State, Nigeria in April, 2017. They were then taken for proper identification and authentication by Mr Chijoke Onyeukwu, a botanist and curator of the Herbarium of the Department of Plant Science and Biotechnology (PSB), University of Nigeria, Nsukka. Voucher numbers were given to the samples as follows: UNH 313 (*Allium cepa*), UNH 214 (*Allium sativum*), UNH 991 (*Zingiber officinale*) and UNH 118 (*Garcinia kola*).

Bacterial strains
Four strains; *Listeria monocytogenes* ATCC 13932 (Gram positive organism), *Escherichia coli* NCTC 10418, *Klebsiella pneumoniae* NCTC 13368 and *Pseudomonas aeruginosa* NCTC 12903 (Gram negative organisms) were obtained from the National Agency for Food and Drug Administration and Control (NAFDAC), Agulu, Anambra State. The strains were collected as nutrient agar slants and immediately transported in an icebox to one of the research labs of Microbiology Department, University of Nigeria, Nsukka where this study was carried out and stored at -20^{o}C. Thereafter, strains were maintained on both nutrient agar slants and broths just before use.

Preparation of extracts
Aqueous, ethanol and hexane extracts of onions, garlic, ginger and bitter kola respectively, were prepared separately. The fresh cloves of onions and garlic were first peeled, sliced and sun dried for twelve days. After drying, the slices of each of the spices or nuts were ground to fine powder separately using a manual blender. The powders obtained were thereafter, macerated with the different solvents in 1000 mL conical flasks for 48 hours in a powder to solvent ratio (g/L) of 1:5 (typically, 100 g of each powder in 500 mL of solvent) according to the method described by Nweze and Eze[13] with modifications. Afterwards, the flasks were incubated at room temperature for 48 hours with shaking at 120 rpm. Filtrates of the extracts (ethanol and hexane), were evaporated at 50°C while the aqueous extracts were evaporated at 80°C in a rotary evaporator. The crude extracts were then stored at 4°C. For easy identification, the extracts were code-named AqAc (Aqueous *Allium cepa*), EtAc (Ethanol *Allium cepa*), HeAc (Hexane *Allium cepa*), AqAs (Aqueous *Allium sativum*), EtAs (Ethanol *Allium sativum*), HeAs (Hexane *Allium sativum*), AqZo (Aqueous *Zingiber officinale*), EtZo (Ethanol *Zingiber officinale*), HeZo (Hexane *Zingiber officinale*), AqGk (Aqueous *Garcinia kola*), EtGk (Ethanol *Garcinia kola*) and HeGk (Hexane *Garcinia kola*) respectively.

Standardization of inoculum
Freshly prepared nutrient broth cultures of the individual bacteria were used as inocula by diluting with sterile saline solution. Each inoculum was thereafter prepared according to the CLSI approved guideline for bacteria and yeasts.[14] This was done as follows: 10mL of the sterile saline solution was transferred to separate test tubes and left to cool. Thereafter, 0.1ml of each of overnight broth cultures of the typed strains *L. monocytogenes*, *E. coli*, *P. aeruginosa* and *K. pneumoniae* were individually dispensed into the separate test tubes containing the solution. The turbidity of each bacterial broth suspension was then adjusted to match the appearance of 0.5 McFarland's standard using sterile normal saline.[14] This served as the standard inocula for the antibacterial activity testing and for the determination of minimum inhibitory concentration of the extracts.

Antimicrobial assay using disc diffusion method
The antimicrobial assay of the three spices and nut was performed using the Kirby-Bauer disc diffusion method as described by Gashe et al.[15] All the experiments were performed under sterile conditions. Aseptically prepared Mueller Hinton agar plates were inoculated separately with 1.5 x 10^8 CFU/mL of each test bacterial culture and then spread evenly on the plate. Sterile discs (6mm diameter) prepared as previously described[16] were dipped aseptically in the different extracts, left to dry and placed over the Mueller Hinton agar plates already seeded with bacterial culture. The plates were left at ambient temperature for 15mins, then inverted and incubated at 37^{o}C for 24 hours. It was observed for zone of inhibition and the inhibition zone diameter (IZD) was measured (in millimeters) using a meter rule and recorded. The end or edge of inhibition zone is where the bacterial growth starts. Antimicrobial assay was performed in triplicates with each bacterial strain.

Determination of minimum inhibitory concentration (single extract assay)
The MIC of the different extracts of onions, garlic, ginger and bitter kola, was determined using the method described by Natta *et al*.[17] with minor modifications. Four test tubes were prepared with the first containing 2 mL of DMSO (dimethyl sulfoxide) and the other three 1mL each. About 0.4 g each of the different extracts was weighed and dispensed first into the first test tube containing 2 mL dilute DMSO. Subsequently, 1mL was taken from the first test tube and dispensed into the second test tube and from the second to third until the last test tube. The concentration of the extracts after dilution therefore ranged from 200 mg/mL, 100 mg/mL, 50 mg/mL and 25 mg/mL, and these were used to determine their individual MIC against the test bacterial strains. Sterile discs were thereafter dipped into each of the extract solutions, allowed to dry and placed on Mueller Hinton agar plates seeded with 1.5 × 10^8 CFU/mL of each of the test bacterial cultures separately. Each experiment was performed in duplicates.

Determination of minimum inhibitory concentration (synergistic assay)
To carry out synergism tests, 0.2 g each of any two extracts being combined (onions and garlic, onions and ginger, onions and bitter kola, garlic and ginger, garlic and bitter kola, ginger and bitter kola) was used, to give a 1:1 ratio. These were added to make up 0.4 g and then diluted as described above in DMSO, to get the different extract concentrations (200mg/mL, 100mg/mL, 50mg/mL and 25mg/mL) respectively. Sterile discs were then dipped into the solutions as above, and allowed to dry before being placed on Mueller Hinton agar plates seeded with 1.5 × 10^8 CFU/ml of each bacterial cultures separately. Each experiment was performed in duplicates.

Statistical analysis
All assays done in the course of this work were in triplicates. Values of inhibition zone diameters were calculated as mean ± SE (standard error) of triplicate measurements obtained for all extracts. This applied to both the single and synergistic assays. The statistical package used was SPSS (Statistical Package for Service Solutions) version 23.

Results and Discussion

The spices and nut *Allium cepa*, *Allium sativum*, *Zingiber officinale* and *Garcinia kola* are plants widely used in different parts of the world as additives and spices to enhance flavors in food and also for medicinal purposes.[7,12,16,18,19] Often called green pharmacy, the use of these type of plants could form a strong base for the development of many interesting drugs with a rich repertoire of properties possessing different mechanisms of actions that could be targeted towards different maladies.[15] The ease of use and far-reaching worldwide spread of most of these spices make them good candidates for such studies. The use of food and spices as medicine is almost as old as

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human history and cuts across different cultures.[18] Moreover, appreciating their importance in that context increases when placed beside the challenge of increasing antimicrobial resistance, and the concomitant decline in the activities of major pharmaceutical companies in the search for new alternatives.[20]

The percentage yield obtained from the extraction of each of the four dried powders of the different spices and nut with the three solvents: water (aqueous), ethanol and hexane, is shown in Table 1. The Table shows that the highest yield was obtained when ginger (*Zingiber officinale*) was extracted with water (AqZO, 17.35%) followed by aqueous garlic (*Allium sativum*, AqAS, 15.91%) while the least was obtained with hexane garlic (*Allium sativum*, HeAS, 1.75%).

A look at the table shows that aqueous solvent showed a consistency unrivalled by the other two. It is not surprising therefore, that the best three yields obtained were aqueous extracts as shown in the yield ratio below:

AqZo> AqAs> AqAc> HeAc> HeGk> AqGk> EtAs> EtAc> EtGk> HeZo> EtZo> HeAs

From the ratio, it is may seem that the least values were obtained with hexane but a closer look at Table 1 shows that it performed better than ethanol in the extraction of three of the plants. These results may partly be explained by the nature of these extractants. It is known that water being the aqueous solvent, is the most polar of solvents and possesses the maximum polarity index of 9.0 while hexane is considered the least polar and therefore the maximum non-polar solvent with a polarity index of 0.0; ethanol is close to water with a polarity index close to the midpoint mark between the two of 5.2.[21] These differences in properties could essentially impart in what the different solvents extract from the different plants.

Another instance of the impact of the different extractants is represented in Table 2 which shows the phytochemical composition of the different extracts. It shows that aqueous extracts of ginger (*Zingiber officinale*) and bitter kola (*Garcinia kola*) have five out of the six phytochemicals assayed for except for tannins and glycosides respectively; aqueous extracts of onions (*Allium cepa*) and ginger (*Allium sativum*) contained four out of the six phytochemicals apart from saponins and tannins and steroids and tannins respectively. Ethanol extracts of all the spices and nuts each had five of the six phytochemicals, and lacked only one of them. Ethanol extracts of onion (ethanolic *Allium cepa*) and ginger (ethanolic *Zingiber officinale*) both lacked steroids; and those of garlic (ethanolic *Allium sativum*) and bitter kola (ethanolic *Garcinia kola*) lacked tannins and glycosides respectively. Lastly, the hexane extracts contained all the phytochemicals except one just like the ethanolic extracts. Hexane onions and garlic (hexane *Allium cepa* and *Allium sativum*) extracts both lacked tannins. On the other hand, hexane ginger and bitter kola (hexane *Zingiber officinale* and *Garcinia kola*) extracts lacked steroids and glycosides respectively.

The differences shown in the class or type of phytochemicals extracted by the different solvents could be explained on the basis of differences in their nature as extractants. Obviously, differences in the properties of the different solvents could impart in what they can extract from the different plants. It is known for example that the extraction principle depends on like dissolving and extracting like, which is the same as saying that only the metabolites that can dissolve in each solvent can be extracted by it from the overall components of the plant material used. This process also known as selective solubility is a basic chemistry concept today.[22,23] These differences obviously have great impact on the phytochemical properties of the different extracts as non-polar solvents typically solubilize mostly lipophilic compounds such as fatty acids, alkanes, waxes, pigments, alkaloids etc., while polar solvents solubilize those like flavonoid glycosides, tannins and some alkaloids among others while those in between like ethanol in this case extract compounds of intermediate polarity which include some alkaloids and flavonoids.[21, 23-25] Therefore, from the phytochemical analysis of the extracts (Table 2), it is seen that they each comprise different phytochemical components such as alkaloids, flavonoids, glycosides, saponins, steroids and tannins with none of them containing all the components. Since the nature and scope of the present work is essentially preliminary and concerned merely with qualitative assessment of the presence or otherwise of the different phytochemical compounds, the detailed analysis of their nature was not carried out, otherwise differences will obviously have been found that could explain their differential inhibitory properties to the test microorganisms which follows below.

The results of the antimicrobial effects of the extracts on the different test microorganisms used are presented in Table 3. The results were different for the different organisms. With respect to *L. monocytogenes*, aqueous extract of *Allium cepa* gave the highest inhibitory activity with an inhibition zone diameter (IZD) mean value of 27.5 ± 0.3, followed closely by the ethanolic extract with a mean IZD value of 20 ± 0.15, the hexane extracts showed no activity. In the case of *Allium sativum*, the extract obtained with ethanol gave a mean IZD value of 24.0 ± 0.2, followed in second place by the aqueous extract whereas the hexane extract showed no inhibitory activity on *L. monocytogenes*. All the three extracts of *Zingiber offinicale* showed some inhibitory activity, although not at the same level as the last two with *L. monocytogenes*. In the case of *Garcinia kola*, only its ethanolextract inhibited the growth of organism with a mean value of 17.5 ± 0.15, while the other two showed no activity. In the case of *E. coli*, ethanol extracts of *Allium cepa* inhibited its growth the most, doing so with the mean IZD value of 30.1 ± 0.3, followed by the aqueous extract, which gave a mean IZD value of 25.5 ± 0.25 against it; hexane extract showed no activity. With *Allium sativum*, only the aqueous and hexane extracts inhibited *E. coli*, but ethanol extracts did not. Regarding *Zingiber officinale*, all three extracts showed some level of inhibition against *E. coli*, with ethanol extract showing the highest mean IZD of just over 19 mm and the least value given as expected by hexane extracts. Only two Garcini *kola* extracts, aqueous and ethanol showed inhibitory activities against *E. coli*, hexane showed none as has become typical. Concerning *P. aeruginosa*, both aqueous and ethanol extracts of *Allium cepa* showed nearly the same mean IZD values of about 17 mm; while the least IZD was again given by its hexane extracts. All three extracts of *Allium sativum* also showed inhibitory activities against *P aeruginosa*. However, whereas expectedly aqueous extract gave the highest IZD value, the second was given atypically by hexane extracts and the least by ethanolic extracts. Extracts from *Zingiber officinale* inhibited *P. aeruginosa* the most, with nearly the same mean IZD values (23 and 22 mm respectively) for aqueous and ethanolic extracts; hexane extracts showed no activity against it. Aqueous extracts of *Garcinia kola* on the other hand gave the most inhibition of *P. aeruginosa* with a mean IZD value of 17 mm, followed by ethanol extracts; hexane extracts gave the least. Finally, regarding *K. pneumonia*, most of the extracts showed no inhibitory activity against it except a few, like aqueous extract of *Allium cepa*, which gave the overall highest inhibition against it (IZD = 28.5 ± 0.15), followed by hexane extracts also of *Allium cepa* (IZD value = 17.5 ± 0.14). None of the three extracts from *Allium sativum* showed activity against *K. pneumoniae*. Only the aqueous extract of *Zingiber officinale* showed some minor inhibitory action against it, both aqueous and hexane extracts of *Garcinia kola* showed minimal inhibitory activities against the organism. On the whole no extract from ethanol showed any form of activity with *K. pneumoniae*. Thus, in terms of susceptibility of the four test organisms: *L. monocytogenes*, *E. coli*, *P. aeruginosa* and *K. pneumoniae*, to the extracts, as an expression of their potency, the highest inhibitory and thus antimicrobial action was exhibited against *E. coli* followed by *L. monocytogenes*. The bacteria *K. pneumoniae* showed very low susceptibility compared to the other bacteria against the plant extracts. Similar results have been reported by others. For example, Ponmurugan and Shyamkumar[8] in their study to determine the antibacterial effect of *Allium sativum* and *Zingiber officinale* rhizomes against multiple drug resistant clinical pathogens found that all the bacterial isolates they used were susceptible to extracts of the two plants with the exception of *Enterobacter* sp. and *K. pneumoniae*. Although clinical isolates were used in their study unlike the present study, this means perhaps that *K. pneumoniae* should be targeted in more studies of this nature to increase the stock of natural products it is susceptible to. Another important difference between the two studies is shown by the fact that unlike in theirs where there was some inhibition albeit very little as extract concentration was increased, there was no form of inhibition as evidenced by nil clearance zones

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obtained with ethanolic extracts of the same plants in the present study. Results presented in Tables 4, 5, 6 and 7 reveal the effect of combinations of the different extracts (synergism) on the inhibition of the growth of *L. monocytogenes*, *E. coli*, *P. aeruginosa*, and *K. pneumoniae* respectively. In Table 4 which is on *L. monocytogenes*, the highest mean IZD value of 35.0 ± 0.1 mm was obtained when the combination of *Allium sativum* and *Zingiber officinale* were extracted with aqueous solution, followed closely by the combination of *Allium sativum* and *Garcinia kola* extracted with ethanol (34.0 ± 0.2 mm). The least inhibition diameter was obtained with the combination of *Allium cepa* and *Allium sativum*, although several other combinations had no effect on *L. monocytogenes*. The result of combinations of the different extracts (synergism) on the inhibition of *E. coli*, Table 5, showed that the highest inhibitory activity against the organism was obtained with the combination again of *Allium sativum* and *Zingiber officinale* when extracted with water (30.0 ± 0.05 mm). It is followed in second place by the combination of *Allium cepa* and *Garcinia kola* extracted with ethanol (27.0 ± 0.18 mm); the same combination when extracted with water gave the overall least inhibitory activity against *E. coli* (7.0 ± 0.1 mm). Lastly only half of the combinations of the spices extracted with hexane showed inhibitory activities against the organism. In the case of Table 6, on *P. aeruginosa*, the highest IZD value of 26.0 ± 0.15 mm was obtained from ethanol extracts of the combination of *Allium cepa* and *Zingiber officinale*; followed by the aqueous extract of the same *Allium cepa* and *Allium sativum* (23.0 ± 0.05 mm). Hexane extracts of the same combination gave the next higher inhibition value (22.0 ± 0.08 mm). Aqueous and hexane extracts of the spice combinations showed no inhibitory activity against *P. aeruginosa*. The last extract combinations result which is against *Klebisella pneumoniae*, presented in Table 7, showed that the highest mean IZD value of 28.5 ± 0.05 mm was obtained with the aqueous extract of the combination of *Allium sativum* and *Garcinia kola*. It was followed somewhat distantly by ethanol extracts of combinations of *Zingiber officinale* and *Garcinia kola* and *Allium sativum* and *Garcinia kola* with values of 21.5 ± 0.18 mm and 21.0 ± 0.25 mm respectively. However, some especially hexane extracts, did not show any inhibitory activity against the microorganism. The results of the synergism showed essentially, that increased antibacterial activity were obtained with combinations of the different solvent extracts of the plants against the test microorganisms. This is a positive highlight of the study. Clearly the results demonstrate that combinations of the extracts showed greater potency and therefore better effects than those obtained singly and without combinations against the test bacteria. The aqueous and ethanol extracts showed the highest activity with inhibition zone diameters of between 30.0 mm to 35.0 mm. The importance of synergism as a potency enhancer in antimicrobial studies is now widely accepted, as synergistic combinations of various antimicrobial agents are considered strategies that are ideal in the management of clinical and multidrug resistant infections.[26] A very recent study indicates that such enhancements are not just observed in antimicrobial studies, that is, with microbially induced health situations but even in metabolic disorders such as diabetes.[27] It is believed that synergism is able to have its positive and more effective impacts, sometimes better than those from purified pharmaceutical compounds, due to the natural synergistic interactions inherent in the many active phytochemical and pharmacological compounds present in the natural plants.[28] Table 8 shows the minimum concentrations of the different extracts needed to inhibit the growth of the different microorganisms. The result shows that in the case of *Allium cepa*, a minimum concentration of 25 mg/mL was required to effect inhibitory activity against most of the microorganisms tested against, whereas for ethanol extracts of the same spice, somewhat higher concentrations (mostly 50 mg/mL) were required. Much higher concentrations were needed to effect inhibitory activities with hexane. For *Allium sativum*, the minimum concentration required to effect inhibitory activities against the test microorganisms ranged mostly between 50 to 100 mg/mL for most of the extracts except against *K. pneumoniae* which showed high resistance. In the case of *Zingiber officinale*, the two lower concentrations of 25 mg/mL and 50 mg/mL were sufficient although in a few cases, the higher concentrations of 100 and 200 mg/mL (especially with hexane), were required to effect microbial inhibition with extracts from the spices. Lastly for *Garcinia kola*, the minimum inhibitory concentrations were somewhat higher, with 25 mg/mL observed only for the ethanol extracts of the nut. For aqueous extract, the minimum required for inhibition of microbial growth was mostly 50 mg/mL. Higher concentrations were as usual required for hexane extracts of the nut. In Table 9, in which is shown the MIC values obtained for the combinations of different solvent extracts of the test plants against the test microorganisms, there were clear variations in the result obtained in most of the cases. Also, the minimum concentration required to inhibit the growth of *K. pneumoniae* and to some extent *P. aeruginosa*, were mostly reduced with the combinations. A few patterns can be observed from the analysis of the minimum inhibitory concentration (MIC) of the different solvent extracts of the test plants on the test organisms. The first is that aqueous extracts in many cases gave the lowest MIC value of 25mg/mL. In some cases, however, ethanol extracts also gave low MIC values.

Table 1: The yield percentage of dried extracts from different spices

Plant material	Aqueous	Ethanol	Hexane
Onion (*Allium cepa*)	12.91%	4.33%	6.27%
Garlic (*Allium sativum*)	15.91%	4.71%	1.75%
Ginger (*Zingiber officinale*)	17.35%	2.33%	3.01%
Bitter kola (*Garcinia kola*)	4.76%	4.24%	5.18%

Table 2: Phytochemical composition of different extracts of the common food spices

Extracts		AC	AS	ZO	GK
Alkaloids	Aqueous	+	+	+	+
	Ethanol	+	+	+	+
	Hexane	+	+	+	+
Flavonoids	Aqueous	+	+	+	+
	Ethanol	+	+	+	+
	Hexane	+	+	+	+
Glycosides	Aqueous	+	+	+	-
	Ethanol	+	+	+	-
	Hexane	+	+	+	-
Saponins	Aqueous	-	+	-	+
	Ethanol	+	+	+	+
	Hexane	+	+	+	+
Steroids	Aqueous	+	+	+	+
	Ethanol	-	+	-	+
	Hexane	+	+	-	+
Tannins	Aqueous	-	-	-	+
	Ethanol	+	-	+	+
	Hexane	-	-	+	+

Key: (+) Present, (-) Absent. AC = *Allium cepa*; AS = *Allium sativum*; ZO = *Zingiber officinale*; GK = *Garcinia kola*.

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Table 3: Growth inhibition (measured as zones of clearance) of different solvent extracts of test plants against the tested microorganisms.

Tested Microorganism	Extracts	AC	AS	ZO	GK
L. monocytogenes	Aqueous	27.5 ± 0.3	13.5 ± 0.15	13.5 ± 0.10	-
	Ethanol	20.5 ± 0.15	24.0 ± 0.2	15.5 ± 0.25	17.5 ± 0.15
	Hexane	-	-	14.2 ± 0.1	-
E. coli	Aqueous	25.5 ± 0.25	16.5 ± 0.15	12.5 ± 0.05	20.0 ± 0.2
	Ethanol	30.1 ± 0.3	-	19.2 ± 0.3	08.4 ± 0.15
	Hexane	-	12.0 ± 0.5	07 ± 0.02	-
P. aeruginosa	Aqueous	17.5 ± 0.14	15.0 ± 0.12	23.0 ± 0.3	17.0 ± 0.31
	Ethanol	17.0 ± 0.3	10.0 ± 0.05	22.1 ± 0.15	11.5 ± 0.05
	Hexane	08.5 ± 0.15	12.5 ± 0.05	-	08 ± 0.02
K. pseudomonas	Aqueous	28.5 ± 0.15	-	09.3 ± 0.15	14.0 ± 0.21
	Ethanol	-	-	-	-
	Hexane	17.5 ± 0.15	-	-	10.0 ± 0.2
Positive control	Tetracycline	40.0 ± 0.05	40.0 ± 0.05	40.0 ± 0.05	40.0 ± 0.05
Negative control	Aqueous	-	-	-	-

Values are diameters of triplicate (n = 3) zones of inhibitions in mm (Mean ± SE); (-) = No activity
AC = *Allium cepa*; AS = *Allium sativum*; ZO = *Zingiber officinale*; GK = *Garcinia kola*

Table 4: Combinations of Extracts of Test Plants with Different Solvents and their Synergistic and other effects against *Listeria monocytogenes*

Extracts	Aqueous	Ethanol	Hexane
AC + AS	08.0 ± 0.01 (Antagonistic)	22.5 ± 0.05 (Additive)	-
AC + ZO	16.5 ± 0.25 (Additive)	29.5 ± 0.15 (synergistic)	18.5 ± 0.05 (synergistic)
AC + GK	25.5 + 0.20 (Additive)	18.5 ± 0.05 (Antagonistic)	10.0 ± 0.05 (synergistic)
AS + ZO	35.0 ± 0.10 (synergistic)	07.5 ± 0.19 (Antagonistic)	- (Antagonistic)
AS + GK	- (Antagonistic)	34.0 ± 0.20 (synergistic)	12.0 ± 0.04 (synergistic)
ZO + GK	16.0 ± 0.30 (Additive)	10.0 ± 0.03 (Antagonistic)	19.0 ± 0.36 (synergistic)

Values are diameters of triplicate zones of inhibitions in mm (Mean ± SE); (-) = No activity AC = *Allium cepa*; AS = *Allium sativum*; ZO = *Zingiber officinale*; GK = *Garcinia kola* AS + ZO gave the highest inhibitory activity against *Listeria monocytogenes* with a mean value of 35.0 ± 0.10.
Synergism is when activity due to the combination is greater than the sum of the actions of each component. The highest synergistic effects occurred in all combinations of *Garcinia kola* and hexane. Additive effects are when the sum of the combination is equal to their individual effects separately, while Antagonism is when the effects of the two combinations is less than the sum of the effects of the two drugs taken independently of each other.

Table 5: Combinations of Extracts of Test Plants with Different Solvents and their Synergistic and other effects against *Escherichia coli*

Extracts	Aqueous	Ethanol	Hexane
AC + AS	24.0 ± 0.35 (Additive)	13.5 ± 0.05 (Antagonistic)	10.0 ± 0.04 (Synergistic)
AC + ZO	19.4 ± 0.26 (Antagonistic)	13.3 ± 0.04 (Antagonistic)	- (Antagonistic)
AC + GK	07.0 + 0.10 (Antagonistic)	27.0 ± 0.18 (Synergistic)	- (Antagonistic)
AS + ZO	30.0 ± 0.05 (Synergistic)	19.4 ± 0.02 (Additive)	23.0 ± 0.05 (synergistic)
AS + GK	18.0 ± 0.04 (Additive)	18.1 ± 0.02 (Synergistic)	24.5 ± 0.15 (Synergistic)
ZO + GK	20.0 ± 0.10 (Additive)	19.0 ± 0.25 (Additive)	- (Antagonistic)

Values are diameters of triplicate zones of inhibitions in mm (Mean ± SE); (-) = No activityAC = *Allium cepa*; AS = *Allium sativum*; ZO = *Zingiber officinale*; GK = *Garcinia kola* AS + ZO gave the highest inhibitory activity against *Escherichia coli* with a mean value of 30.0 ± 0.05 This table shows that hexane extracts has more synergistic effect than others.
Synergism is when activity due to the combination is greater than the sum of the actions of each component. The highest synergistic effects occurred in all combinations of *Garcinia kola* and hexane.
Additive effects are when the sum of the combination is equal to their individual effects separately, while Antagonism is when the effects of the two combinations is less than the sum of the effects of the two drugs taken independently of each other.

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Table 6: Combinations of Extracts of Test Plants with Different Solvents and their Synergistic and other effects against *Pseudomonas aeruginosa*

Extracts	Aqueous	Ethanol	Hexane
AC + AS	23.0 ± 0.05 (Synergistic)	13.2 ± 0.05 (Additive)	22.0 ± 0.08 (Synergistic)
AC + ZO	14.2 ± 0.01 (Additive)	26.0 ± 0.15 (Additive)	21.0 ± 0.18 (Synergistic)
AC + GK	21.0 + 0.15 (Synergistic)	8.0 ± 0.11 (Antagonistic)	18.0 ± 0.02 (Synergistic)
AS + ZO	14.0 ± 0.08 (Additive)	20.0 ± 0.05 (Additive)	- (Antagonistic)
AS + GK	- (Antagonistic)	21.1 ± 0.05 (Synergistic)	13.5 ± 0.17 (Additive)
ZO + GK	- (Antagonistic)	14.5 ± 0.15 (Antagonistic)	- (Antagonistic)

Values are diameters of triplicate zones of inhibitions in mm (Mean ± SE); (-) = No activity AC = *Allium cepa*; AS = *Allium sativum*; ZO = *Zingiber officinale*; GK = *Garcinia kola* AS + ZO gave the highest inhibitory activity against *Pseudomonas aeruginosa* with a mean value of 26.0 ± 0.15. Aqueous extracts of AC + AS caused the highest synergism of 23.0 ± 0.05 followed by AC + AS from hexane
Synergism is when activity due to the combination is greater than the sum of the actions of each component. The highest synergistic effects occurred in all combinations of *Garcinia kola* and hexane.
Additive effects are when the sum of the combination is equal to their individual effects separately, while Antagonism is when the effects of the two combinations is less than the sum of the effects of the two drugs taken independently of each other.

Table 7: Combinations of Extracts of Test Plants with Different Solvents and their Synergistic and other effects against *Klebsiella pneumoniae*

Extracts	Aqueous	Ethanol	Hexane
AC + AS	07.0 ± 0.05 (Antagonistic)	14.2 ± 0.05 (Synergistic)	19.0 ± 0.05 (Synergistic)
AC + ZO	18.5 ± 0.05 (Additive)	13.0 ± 0.10 (Synergistic)	- (Antagonistic)
AC + GK	11.8 + 0.03 (Antagonistic)	08.0 ± 0.05 (Synergistic)	13.5 ± 0.04 (Additive)
AS + ZO	- (Antagonistic)	-	14.5 ± 1.5 (Synergistic)
AS + GK	28.5 ± 0.05 (Synergistic)	21.0 ± 0.25 (Synergistic)	- (Antagonistic)
ZO + GK	- (Antagonistic)	21.5 ± 0.18 (Synergistic)	- (Antagonistic)

Values are diameters of triplicate zones of inhibitions in mm (Mean ± SE); (-) = No activity AC = *Allium cepa*; AS = *Allium sativum*; ZO = *Zingiber officinale*; GK = *Garcinia kola* AS + GK gave the highest inhibitory activity against *Klebsiella pneumoniae* with a mean value of 28.0 ± 0.05. All ethanol extracts except AS + ZO showed synergistic effect on *Klebsiella pneumonia*.
Synergism is when activity due to the combination is greater than the sum of the actions of each component. The highest synergistic effects occurred in all combinations of *Garcinia kola* and hexane.
Additive effects are when the sum of the combination is equal to their individual effects separately, while Antagonism is when the effects of the two combinations is less than the sum of the effects of the two drugs taken independently of each other.

Table 8: Minimum Inhibitory Concentration of Different Solvent Extracts of Test Plants against Test Organisms

	L. monocytogenes	*E. coli*	*P. aeruginosa*	*K. pseudomonae*
Allium cepa				
Aqueous	25 mg/mL	25 mg/mL	25 mg/mL	50 mg/mL
Ethanol	100 mg/mL	50 mg/mL	50 mg/mL	100 mg/mL
Hexane	200 mg/mL	50 mg/mL	200 mg/mL	-
Allium sativum				
Aqueous	100 mg/mL	50 mg/mL	50 mg/mL	200 mg/mL
Ethanol	100 mg/mL	50 mg/mL	100 mg/mL	-
Hexane	50 mg/mL	100 mg/mL	100 mg/mL	-
Zingiber officinale				
Aqueous	25 mg/mL	100 mg/mL	25 mg/mL	100 mg/mL
Ethanol	50 mg/mL	100 mg/mL	25 mg/mL	200 mg/mL
Hexane	100 mg/mL	200 mg/mL	-	-
Garcinia kola				
Aqueous	100 mg/mL	200 mg/mL	50 mg/mL	50 mg/mL
Ethanol	25 mg/mL	25 mg/mL	100 mg/mL	-
Hexane	100 mg/mL	100 mg/mL	200 mg/mL	100 mg/mL

Key: (-) No inhibition. The above aqueous extract of *Allium cepa* showed the strongest activity against the test organisms with the best MIC as 25mg/mL. The highest MIC for all the test organisms was recorded for hexane extracts.

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Table 9: Minimum Inhibitory Concentrations of Synergistic combination of Different Solvent Extracts of Test Plants against Test Microorganisms

	L. monocytogenes	***E. coli***	***P. aeruginosa***	***K. pneumoniae***
AQUEOUS				
AC + AS	50 mg/mL	25 mg/mL	25 mg/mL (synergistic)	
AC + ZO	25 mg/mL	50 mg/mL	100 mg/mL	100 mg/mL
AC + GK	25 mg/mL	100 mg/mL	25 mg/mL (synergistic)	100 mg/mL
AS + ZO	50 mg/mL (synergistic)	25 mg/mL (synergistic)	100 mg/mL	-
AS + GK	50 mg/mL	50 mg/mL	200 mg/mL	25 mg/mL (synergistic)
ZO + GK	100 mg/mL	100 mg/mL	50 mg/mL	200 mg/mL
ETHANOL				
AC + AS	50 mg/mL	100 mg/mL	100 mg/mL	100 mg/mL
AC + ZO	50 mg/mL (synergistic)	100 mg/mL	25 mg/mL (synergistic)	100 mg/mL
AC + GK	100 mg/mL	25 mg/mL (synergistic)	200 mg/mL	200 mg/mL
AS + ZO	100 mg/mL	50 mg/mL	50 mg/mL	-
AS + GK	50 mg/mL (synergistic)	50 mg/mL (synergistic)	50 mg/mL (synergistic)	50 mg/mL (synergistic)
ZO + GK	50 mg/mL	50 mg/mL	100 mg/mL	50 mg/mL (synergistic)
HEXANE				
AC + AS	100 mg/mL	100 mg/mL	50 mg/mL (synergistic)	50 mg/mL (synergistic)
AC + ZO	200 mg/mL	200 mg/mL	50 mg/mL (synergistic)	200 mg/mL
AC + GK	100 mg/mL	25 mg/mL (synergistic)	100 mg/mL	100 mg/mL
AS + ZO	50 mg/mL	25 mg/mL (synergistic)	100 mg/mL	200 mg/mL
AS + GK	200 mg/mL	100 mg/mL	-	-
ZO + GK	50 mg/mL (synergistic)	200 mg/mL	200 mg/mL	-

Key: (-) No inhibition AC = *Allium cepa*; AS = *Allium sativum*; ZO = *Zingiber officinale*; GK = *Garcinia kola*

The results perhaps indicate that the four plants and their extracts especially the aqueous and ethanol-based ones may possess broad spectrum antimicrobial properties against the test microorganisms. More studies will obviously have to be done to situate these conjectures and claims although it does not take much to see that because of their greater polarity, these two solvents in the main typically extracts more secondary and allied metabolites that could have more inhibitory effects against microorganisms. Studies by investigators like Bussmann *et al*.[29] and Mostafa *et al*.[30] whose work were mostly done using aqueous and ethanol-based solvents have shown the consistency of these two solvents in achieving the best extractions and giving the least MICs with most plants. In a way this demonstrates their ability to penetrate the complex microscopic depths of most plants through forming strong bonds such as hydrogen bonds that can counteract and disrupt lipid-protein association[23]and thus dissolves and extract them.

Conclusion

In this study different extracts were obtained using water, ethanol and hexane as solvents from the universally used spices *Allium cepa, Allium sativum and Zingiber officinale* and the nut *Garcinia kola*. The extracts which were used at varying concentrations, showed good

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inhibitory capabilities and potency against some selected bacterial strains. Aqueous solvent extracted more metabolites and also required less concentration (MIC) to exhibit its inhibitory actions compared to the other two solvents. In terms of susceptibility to the extracts, *E. coli* and *L. monocytogenes* showed greater susceptibility, than *P. aeruginosa* and *K. pneumoniae* which showed greater resistance to the extracts. The study is a further demonstration of the many positive attributes, including antimicrobial properties, of many plant-based foods and spices used all over the world. Further future works with the extracts will focus on using much lower concentrations together with more solvents in different combinations and also further layers of purifications so as to further characterize and narrow the metabolites to their specific nature and properties.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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