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PHYTOCHEMICAL ASSAY AND ANTIBACTERIAL EFFECTS OF COMBINED SEED EXTRACTS OF. *Aframomum melegueta* K. Schum AND *Garcinia kola* Heckel ON *Salmonella typhi* and *Klebsiella pneumoniae*

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ABSTRACT

The rising cases of drug resistant microbial strains, due to adaptation and continuous use of orthodox medicines, as well as the myriads of attendant associated challenges had necessitated attention to ethno-botanicals options. Consequently, the study on secondary metabolite assay and antibacterial effects of seed extracts of *Aframomum melegueta* K. Schum, *Garcinia kola* Heckel and their combined extracts on *Salmonella typhi* and *Klebsiella pneumoniae* was conducted, using standard methods. The bioactive compounds assayed revealed the presence of flavonoids, alkaloids, tannins, saponins, phenols by both the methanolic and aqueous extractants from all the samples. Glycosides and terpenoids were present only in methanolic and aqueous extractants respectively, while anthraquinones and courmarins were present only in the hot aqueous extractant. The average inhibitory zones were higher with methanolic extract (ranging from 11.0-13.0mm) than those of the aqueous(2.9-4.0mm). The positive control drug (ciproflaxocin) had 28.2 and 29.2mm for *S. typhi and K pneumonia*. The minimum inhibitory concentration (MIC) ranged from 12.5 – 200mg/ml and 50 – 400mg/ml for *K. pnemoniae* and *S. typhi* respectively. All the aqueous extracts had turbidity at the highest test concentration of 400mg/ml. However, the minimum bactericidal concentration (MBC) ranged from 100 – 200mg/ml and 50mg/ml for *S. typhi.* and *K. pnemoniae*. The antimicrobial power (AP) otherwise described as MBC/MIC ratio showed all extracts of test plants indicated bactericidal effects except methanolic *G. kola* (GMetOH) and combined *A. melegueta* and *G. kola*(A+GMetOH) extracts gave bacteriostatic effects against *K. pneumoniae* and *S. typhi* respectively. The fractional inhibitory concentration index(FICI or ∑FIC) showed that both combined extracts(A+GMetOH and A+GH_2O) gave an indifferent effects on *S. typhi,* only A+GMetOH indicated antagonistic effects against *K. pnemoniae*. This findings revealed some potential preliminary therapeutic properties of the test plants, requiring further investigations for optimal quality integrity, development and control.

Keywords: Secondary metabolite, Methanolic, Aqueous, Antibacterial, Bacteriostatic

Introduction

The continuous use of orthodox medicines for the cure of clinical ailments has met with a number of attendants setbacks ranging from development of drug resistance by strains by microbial agents (Mayrink-Assis *et al*., 2017), high production and procurement costs, higher records of incidences of side effects, as compared with any herbal alternative medication, depend on the drug in question (Nisar *et al*., 2017). Others include restricted mode of administration as well as inability to exhibit wider targets, unlike the traditional Herbal options (Sam, 2019). Consequent upon these, the need for natural alternatives with relatively little or no side effects, cheaper,

readily available ethno-botanical options became imperative.

Plants constitute an important source of active ingredients which differ widely in terms of structure and therapeutic properties. There has been a renewed efforts in research bordering on developing therapeutics, including antimicrobials, from plant bioresources (Eneh *et al.*, 2017). The medicinal values of the ethnobotanicals lie in the quantitative and qualitative properties of the bioactive constituents,(also known as secondary metabolites) such as alkaloids, saponnins, flavonoids, tannins and phenolic compounds (Doherty, 2010; Nas *et al*., 2017). These phytochemicals present in parts of the plant such as seed, leaves bark and root are toxic to microbial cells. Most modern medicines depend on herbal extracts from plants as fundamental source of therapeutic ingredients, which are pools of potential antimicrobial compounds for pharmaceutical need (Nas *et al*., 2017).

Aframomum melegueta is a member of the ginger family - *Zingiberaceae* also known as Alligator pepper. It is a herbaceous perennial plant having a pungent peppery flavour. *A. melegueta* possess potent anti-inflammatory activity with favorable gastric tolerability profile and valued for its warming and digestive properties and antimicrobial activities (Doherty *et al.,* 2010). *A. melegueta* seed cures dysentery; serve as a sedative against toothache, anti-rheumatism, migraine and fever (Dokosi, 1998).

Garcina kola from the family guttiferae is highly valued in Nigeria for its edible nuts. It exhibits very potent pharmacological activities such as antioxidant, antibacterial, antifungal, antiviral and anti-inflammatory properties (Adegboye *et al.,* 2008). The seeds of *G. Kola* have pharmacological uses in treating coughs, throat infections, bronchitis, hepatitis (inflammation of the liver), liver disorders (Farombi *et al.,* 2005). The caffeine in the nuts also acts as a bronchodilator, expanding the bronchial air passages, hence Kola nuts are often used to treat whooping cough and asthma. Ezeanya and Daniel (2013), reported antimicrobial effects of dried leaf and seed extracts of *G. kola* against some clinical microbial isolates.

S. typhi is a bacterium reported to be responsible for the death of over 600,000 people annually all over the world (Falkow *et al*., 2004). It is transmitted through food and water, causing high fever, headache, nausea, loss of appetite, diarrhea, enlargement of the spleen and strong resistance to the innate immune response system (Falkow *et al*., 2004). Increase in multi-drug resistance, adaptation and survival in the *S. typhi* population are consequences of continuous exposures to chemically based medicines (Philippa *et al*., 1998). *Klebsiella pneumoniae* is *a* Gram negative ubiquitous bacterium, recognized over 100 years ago as a cause of community acquired pneumonia and is the opportunistic pathogen that can cause pneumonia, urinary tract infections, and bacteremia (Wu *et al.,* 2012).

Many attempts have been made at assessing the combined effects of different plant species with respective conventional antibiotics against specific clinical isolates (Stefanovic *et al*., 2011; Javed *et al*., 2012; Tariq *et al*., 2014). These attempts were to avoid antibacterial resistance and romote the discovery of novel drugs (Al-Saiym *et al*., 2015). Although different parts of or plant species are being combined for optimal therapeutic efficacy. This is with the view of harnessing their active components to obtain synergistic or additive effects, and phytomedicinal efficiency (Van Vuuren and

Viljoen, 2011). However, only few reported cases exist. This work therefore assesses the phytochemical assay and antibacterial effects of combined seed extracts of *Garcinia kola* and *Aframomum melegueta* on *salmonella typhi* and *klebsiella pneumoniae.*

Materials and Methods

Collection and Preparation of Plant Materials

The seeds of *Garcinia kola* and *Aframomum melegueta* were procured from Yan goro market, Jos - Plateau state, Nigeria. They were taken for identification at the College Herbarium of Federal College of Forestry, Jos, Nigeria. They were carefully washed with distilled water and air-dried under shade for two weeks to maintain compositional integrity (Ezeanya and Daniel, 2013). The seeds were pulverized, using Thomas Wiley Laboratory mill model 4, and stored in the desiccator until use.

Source of Clinical Isolates

The test organisms, *Salmonella typhi* and *Klebsiella pneumonia* (already characterized) were sourced from Ahmadu Bello University Teaching hospital, Shika-Zaria, Kaduna state, Nigeria. They were kept at Microbiology laboratory, of Nigerian Institute of Leather and Science Technology, (NILEST), Samaru, Zaria.

Methods of extraction Aqueous Extraction (Hot water)

Standard methods of Okigbo and Omodamiro (2006) was modified and adopted. 30g of the pulverized seeds of both test plant materials were separately soaked in 300ml hot water boiled for 30 minutes and kept for 24 hours, thereafter filtered using sieve and then cotton wool to obtain fine filtrates. The filtrates were concentrated by evaporation process using hot air oven for 24 hours and the resultant extracts were collected in an air-tight bottles and kept in desiccators until use. (Ardzard *et al.*,2009).

Soxhlet extraction (methanolic)

Thirty grams (30g) of the samples were separately extracted continuously for 3 hours with 70% methanol at 70^{o}C, using the soxhlet apparatus. The excess solvent was recovered using the same procedure. The extracts were concentrated in an evaporating dish using water-bath. The extracts were weighed and used for phytochemical screening and antimicrobial analysis (Sukhdev *et al.,* 2008).

Sterilization and Disinfection

All the glass wares and test tubes used were sterilized by autoclaving at 121^{o}C for 15 minutes. Wire loop and cork-borer were sterilized by flaming in Bunsen burner until they become red hot, and cooled before use. The work bench surfaces were disinfected using dettol, this was done by sprinkling the disinfectant on the surface and wiped with cotton wool. Hands were washed with soap and dettol, repeatedly rinsed with clean water before and after work (Ardzard *et al.,* 2009).

Standardization of Test Organisms

McFarland Nephelometer methods (Albert *et al*, 1991), were used to standardized all test inoculums. The protocol for preparing this solution gives rise to turbid solutions at room temperature and were kept on the work bench for use. This involved pipetting 0.5ml of already prepared nutrient broth into a sterile test tube aseptically inoculated with pure cultures of the particular test organism until the bacterial suspension match the turbidity of the standard solution. And this bacterial suspension corresponds to 1.5 x 10^5 / bacterial suspension per milliliter.

Preparation of Culture Media

The culture medium, nutrient agar, was prepared according to manufactures instruction (Oxoid CM003, 28g in 1.0 litre of distilled water). 28g of nutrient agar was weighed into a conical flask, 1000ml of distilled water was added and capped. The medium was shaken to dissolution and sterilized at 121°C for 15 minutes, as described by Ardzard *et al.* (2009).

Determination of Antibacterial Activity

The antibacterial effects of aqueous and methanol seed extracts of *Garcinia kola, Aframomum melegueta* and combined mixture of these extracts were determined using agar well diffusion method according to Perez *et al*, (1990) and Ahmed and Beg (2001). Nutrient agar plates were swab with the broth culture of the respective bacteria isolates using sterile wire loop, allowed to diffuse at room temperature for 2 hours and incubated at 37°C for 24 hours. Diameters of the inhibition zones were measured. The antibacterial activities were expressed as the mean diameter zone of inhibition (in millimeter), measured with a transparent ruler.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC effects of aqueous and methanol seed extracts of *Garcinia kola, Aframomum melegueta* and the combined mixture, were determined as the lowest concentration of the various extracts that inhibit the growth of the isolates. The bacteriological peptone was poured into test tubes in appropriate volumes. The tubes were thoroughly mixed and incubated at 37°C for 24 hours, thereafter examined for visible turbidity. Positive tests were tubes with visible turbidity, indicating microbial growth while negative tests were undisturbed (no turbidity), without microbial growth. The minimum inhibitory concentration (MIC) was reported as the lowest concentration that prevented visible growth (Cheesbrough, 2000).

Determination of Minimum Bactericidal Concentration (MBC)

The MBC effects of aqueous and methanol seed extracts of *Garcinia kola, Aframomum melegueta* and the combined mixture was determined by sub-culturing all the tubes in each set in without visible turbidity observed during the tests for MIC. A loop full of the contents of the tubes showing no microbial growth were sub-cultured by streaking over the surface of already set nutrient agar plates without extracts. The plates were incubated at 37°C for 24hours. The MBC was recorded as the lowest concentration which no growth was observed after sub-culturing. All plates showing no growth on the nutrient agar indicated bactericidal effect of the extracts concentration (Cheesbrough, 2000).

Antimicrobial Indices

The effect of an antibiotic substance on a microorganism is measured by a number of indices relative to its minimum inhibitory Concentration (MIC) and minimum Bactericidal Concentration (MBC). The ratio of MBC/MIC describes the antibiotic power (AP) (Yehouenou, 2012). According to Noumedem *et al.* (2013), If the ratio MBC/MIC is ≤4, the effect considered bactericidal, but the ratio is > 4, the effect is described as bacteriostatic.

The fractional inhibitory concentration indices (FIC) The FIC index expresses the interaction of two or more agents in which the concentration of each test agent in combination is expressed as a fraction of the concentration that would produce the same effect when used independently (Berenbaum, 1978). It was calculated as the MIC of the combination divided by the MIC of each

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individual component extract, while the FIC index (FICI) was evaluated as the sum of each component FIC in a combination. FICI is synergistic, if value is ≤ 0.5, additive (0.5–1.0), indifferent (1–4.0) or antagonistic (≥4.0) (Schelz *et al*., 2006; Iten, 2009).

The FIC Index for each combination of antimicrobial agents was calculated using the following formula(Eq. 1):

$$\text{FIC Index} = \frac{\text{MIC extract 1 in combination}}{\text{MIC extract 1 alone}} + \frac{\text{MIC extract 2 in combination}}{\text{MIC extract 2 alone}} \quad \text{……..(Eq. 1)}$$

Results

The Phytochemical Constituents of Seed extracts of test plants

The Phytochemical screening of seed extracts of *Aframomum melegueta*, *Garcinia kola* and the combined extracts showed the presence of alkaloids from both extractants, with higher contents of flavonoids and phenols in the hot aqueous than the methanolic. Anthraquinones and coumarins tested positive only in hot aqueous solvent while steroids were present only with the methanolic. Terpennoids tested highly positive with methanolic extracts of *A. melegueta*, absent only with the hot aqueous combined extracts of the test plants. The glycosides were present in all, but the hot aqueous extract of *A. melegueta.* Tannins were present in all but higher only in *G. kola* and combined extracts. Saponins were highly present in all (Table 1). Generally, the hot aqueous solvent showed a better extractant than the methanol.

Sensitivity Tests

The sensitivity of the test microbial isolates to the plant extracts varied with microbes as well as the nature of the extractants. The *Salmonella typhi* recorded (11.0, 12.0 and 11.5mm) and (3.0, 4.0, 3.6mm) as average inhibition zones (AIZ) for methanolic and aqueous extracts of *Aframomum melegueta*, *Garcinia kola* and the combined extract respectively. Conversely, *Klebsiella pneumonia* gave (13.0, 12.0 and 12.5mm) and (3.0, 3.3, 2.9 and 29.2mm mm) as average inhibition zones (AIZ) for methanolic and aqueous extracts of *Aframomum melegueta*, *Garcinia kola* and the combined extract. The control antibiotics (Ciprofloxacin) recorded 28.2 and 29.2 as AIZ for *S .typhi and K. pneumonia* respectively (Figure 1). The effects of the test drug and the seed extracts were significantly different ($p<0.05$) on the sensitivity of the test microbes (Figure 1).

Methanolic extracts of *A. melegueta* (AMetOH), *Garcinia kola* (GMetOH) and the combined extracts (A+GMetOH) recorded 200mg/ml, 50mg/ml and 50mg/ml as minimum inhibitory concentration (MIC), against *Salmonella typhi.* While the aqueous extracts of *A. melegueta* (AH_2O), *G. kola* and (GH_2O) and the combined extracts ($A+GH_2O$) had 400mg/ml each for *S. typhi*. Conversely, for *Klebsiella pneumoniae*, AMetOH, GMetOH and A+GMetOH gave 100mg/ml, 12.5mg/ml and 50mg/ml as MIC values, while AH_2O, GH_2O and $A+GH_2O$ had 200mg/ml each, for the test organisms (Table 2). These observations clearly showed that the sensitivity of the test organisms to plant extract depended on the organism as well as type of solvent (Table 2).

Minimum bactericidal concentrations (MBC) against *Salmonella typhi* were 200mg/ml, 100mg/ml and 200mg/ml for AMetOH, GMetOH and A+GMetOH respectively, while AH_2O, GH_2O and $A+GH_2O$ gave >400mg/ml each. On the other hand, *K. pneumoniae* recorded 50mg/ml each for AMetOH, GMetOH and A+GMetOH, while AH_2O, GH_2O and $A+GH_2O$ had >400mg/ml each as MBC values. These observations clearly

showed that the sensitivity of the test organisms to plant extract depended on the organism as well as type of extractant used (Table 3). The importance of the nature and solvent types as determinants of microbial sensitivity to the test plant extracts was indicative.

Antimicrobial Parameters and Indices

The antibiotic power, AP (MBC/MIC ratio), evaluated showed that AMetOH, AH_2O, GMetOH, GH_2O, and A+GH_2O with 0.5, >1.0, 2.0, >1.0 and >1.0 respectively were bacteriocidal against *S. typhi*. While A+GMetOH which recorded 4.0 showed bacteriostatic effects against the microbe. Conversely, MBC/MIC ratio of 0.5, >2.0, >2.0, >1.0 and >2.0 for AMetOH, AH_2O, GH_2O, A+GMetOH, A+GH_2O respectively, were considered bacteriocidal against *K. pnemoniae*, while GMetOH with a value of 4.0 had a bacteriostatic effects on *K. pnemoniae.* The fractional inhibitory concentration indices (FICI) evaluated revealed that the methanolic combined extracts (A+GMetOH) and aqueous (A+GH_2O) gave 1.25 and 2.0 respectively for *S. typhi*. However, *K. pnemoniae* recorded 2.0 and 4.5 for (A+GMetOH) and aqueous (A+GH_2O) (Table 4).

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Table 1: Secondary Metabolite compositions Obtained from the Test Plants Based on the Extractants

Extract		*Aframomum melegueta*(X)		*Garcinia kola* (Y)		Combined Extract (X+Y)	
		Extractant		Extractant		Extractant	
S/N	Phytochemicals	Methanol	Hot Aqueous	Methanol	Hot Aqueous	Methanol	Hot Aqueous
1	Alkaloids	+	+	+	+	+	+
2	Flavonoids	++	++	+	++	+	++
3	Tannins	+	+	++	++	++	++
4	Saponins	++	++	++	++	++	++
5	Glycosides	−	++	+	++	+	++
6	Terpenoids	+	++	+	−	+	+
7	Anthraquinones	−	+	−	+	−	+
8	Phenols	+	++	+	++	+	++
9	Steroids	+	−	+	−	+	−
10	Coumarins	−	++	−	++	−	++

− = absence of phytochemical, + = presence of phytochemical, ++ = strong presence of phytochemical.

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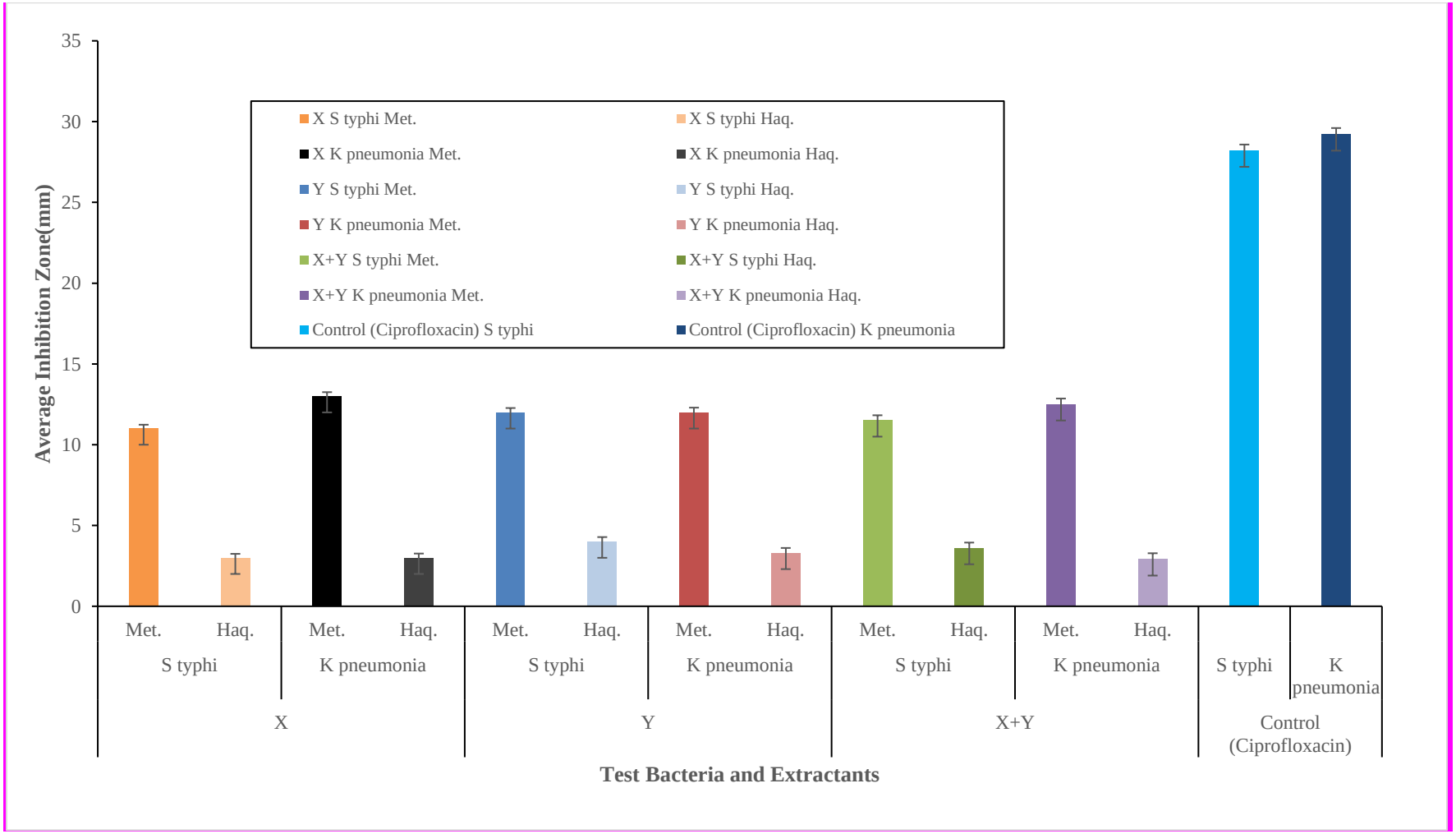

Figure1: Effects of *Aframomum melegueta* seed extracts, *Garcinia kola* seed powder. Combined seed powder on Average Inhibition Zones of *S. typhi and K. pneumoniae*; Where: X = *Aframomum melegueta* seed powder; Y = *Garcinia kola*

seed powder; X+Y = Combined seed powder; Met = Methanol; Haq. = Hot aqueous

Table 2: Effects of *Aframomum melegueta* seed extracts, *Garcinia kola* seed powder. Combined seed powder on Minimum Inhibitory concentration *S. typhi and K. pneumoniae*

		Concentration of Extract(mg/ml)									
	Extract	400	200	100	50	25	12.5	6.25	3.125	MIC	∑FIC
S. typhi	AMetOH	−	−	+	+	+	+	+	+	200	
	AH_2O	−	+	+	+	+	+	+	+	400	
	GMetOH	−	−	−	−	+	+	+	+	50	
	GH_2O	−	+	+	+	+	+	+	+	400	
	A+GMetOH	−	−	−	−	+	+	+	+	50	1.25
	A+GH_2O	−	+	+	+	+	+	+	+	400	2.00
K. pnemoniae	AMetOH	−	−	−	+	+	+	+	+	100	
	AH_2O	−	−	+	+	+	+	+	+	200	
	GMetOH	−	−	−	−	−	−	+	+	12.5	
	GH_2O	−	−	+	+	+	+	+	+	200	
	A+GMetOH	−	−	−	−	+	+	+	+	50	4.50
	A+GH_2O	−	−	+	+	+	+	+	+	200	2.00

MIC = Minimum Inhibitory Concentration; - = No visible growth; + = visible growth; AMetOH= Methanolic seed extract of *Aframomum melegueta;* AH_2O = Aqueous seed extract of *Aframomum melegueta;*GMetOH = Methanolic seed extract of *Garcin ia kola;* GH_2O = Aqueous seed extract of *Garcinia kola;* A+GMetOH = Methanolic extract of combined seeds of *Aframomum melegueta* and *Garcinia kola;* A+GH_2O = Aqueous extract of combined seeds of *Aframomum melegueta* and *Garcinia kola.*

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Table 3: Effects of *Aframomum melegueta* seed extracts, *Garcinia kola* seed powder. Combined seed powder on Minimum Bactericidal Concentration *S. typhi and K. pneumoniae*

		Concentration of Extract(mg/ml)								
Microbe	Extract	400	200	100	50	25	12.5	6.25	3.125	MBC
S. typhi	AMetOH	−	−	+	+	+	+	+	+	200
	AH_2O	+	+	+	+	+	+	+	+	>400
	GMetOH	−	−	−	+	+	+	+	+	100
	GH_2O	+	+	+	+	+	+	+	+	>400
	A+GMetOH	−	−	+	+	+	+	+	+	200
	A+GH_2O	+	+	+	+	+	+	+	+	>400
K. pnemoniae	AMetOH	−	−	−	−	−	+	+	+	50
	AH_2O	+	+	+	+	+	+	+	+	>400
	GMetOH	−	−	−	−	−	+	+	+	50
	GH_2O	+	+	+	+	+	+	+	+	>400
	A+GMetOH	−	−	−	−	−	+	+	+	50
	A+GH_2O	+	+	+	+	+	+	+	+	>400

MBC = Minimum Bactericidal Concentration; - = No visible growth; + = visible growth; AMetOH= Methanolic seed extract of *Aframomum melegueta;* AH_2O = Aqueous seed extract of *Aframomum melegueta;* GMetOH = Methanolic seed extract of *Garcinia kola;* GH_2O = Aqueous seed extract of *Garcinia kola;* A+GMetOH = Methanolic extract of combined seeds of *Aframomum melegueta* and *Garcinia kola;* A+GH_2O = Aqueous extract of combined seeds of *Aframomum melegueta* and *Garcinia kola.*

Table 4: Antimicrobial Parameters and Indices

Microbe	Property	AMetOH	AH_2O	GMetOH	GH_2O	A+GMetOH	A+GH_2O
S. typhi	MIC	200	400	50	400	50	400
	MBC	200	>400	100	>400	200	>400
	MBC/MIC	0.5	>1.0	2.0	>1.0	4.0	>1.0
	∑FIC					1.25	2.0
K. pnemoniae	MIC	100	200	12.5	200	50	200
	MBC	50	>400	50	>400	50	>400
	MBC/MIC	0.5	>2.0	4.0	>2.0	1.0	>2.0
	∑FIC					4.5	2.0

MIC = Minimum Inhibitory Concentration; MBC = Minimum Bactericidal Concentration; MBC/MIC = Ratio of Minimum Bactericidal Concentration to Minimum Inhibitory Concentration; ∑FIC = Fractional Inhibitory Concentration Index; AMetOH= Methanolic seed extract of *Aframomum melegueta;* AH_2O = Aqueous seed extract of *Aframomum melegueta;* GMetOH = Methanolic seed extract of *Garcinia kola;* GH_2O = Aqueous seed extract of *Garcinia kola;* A+GMetOH = Methanolic extract of combined seeds of *Aframomum melegueta* and *Garcinia kola;* A+GH_2O = Aqueous extract of combined seeds of *Aframomum melegueta* and *Garcinia kola*.

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Discussion

The presence of bioactive constituents to a large extent is solvent dependent, as observed by Doherty *et al.*, (2010), who reported the presence of alkaloids, tannins, saponin, steroids, cardiacglycoside, flavonoid, terpenoids and phenol in *Aframomum melegueta*. Idris and Abubakar (2016), corroborated these observations and opined that the metabolites would determine their effectiveness. According to Jeruto *et al*. (2017), the presence of these phytochemcals in the test plants supported their use and efficacy as antimicrobial agents. These bioactive substances effect different metabolic changes, which may be lethal to the microbes. Shaimaa (2014), reported that tannins showed the capacity for bacteria lethal protein complex, while saponins have been reported to alter the permeability, structure and function of cell membranes, leading to their destruction (Arabski *et al*, 2012). Maria *et al*, (2013), posited that phenols exhibit antioxidant and enzyme inhibitory tendencies, while flavonoids have been noted for inhibition of nucleic acid and cell wall synthesis (Mishra *et al.*, 2009). Alkaloids is rated the most efficient therapeutically significant phytocheical (Bamidele, 2019). This is sequel to their capacity to effect structural and genetic imbalance, bacterial DNA cell wall damage and lysing, referred to as intercalation (Firempong *et al*., 2016).

The antimicrobial effects of the plant extracts singly and in combination have been evaluated on the basis of sensitivity responses of the test microbial isolates to plant extracts. The sensitivity tests were based on inhibition zones, bactericidal and bacteriostatic parameters. According to Sarjono *et al.* (2019), The zones of inhibition were categorized and benchmarked, such that a range of inhibitory zone of 20.0 mm or more, 10.0 – 20.0 mm, 5.0 – 10.0 mm and < 5.0 mm were described as very strong, strong, moderate and weak antibacterial potential respectively. In this study, all the methanolic extracts showed strong antimicrobial potentials, with average inhibiting zones (AIZ) ranging from 11.5 – 13.0mm (Figure 1), irrespective of the plants and microbes. Conversely, all the hot aqueous extracts with AIZ ranging from 2.9 - 4.0 mm indicated of *A. melegueta*(13.0mm), *G. kola* (12.0mm) showed strong antibacterial weak antibacterial potential respectively. These findings corroborated the positions of Ogodo *et al* (2017), that the organic base (ethanol) exhibited higher antibacterial activity than the aqueous. Similarly, Idris and Abubakar (2016), reported a lower ZOI with aqueous solvent than the methanol extract. The control drug (ciprofloxacin) had the highest AIZ of 28.2 and 29.2mm for *S. typhi* and *K. pnemoniae* which supported the findings of Nas *et al* (2017).

Lower values of minimum inhibitory concentrations (MIC) were generally observed for *Klebsiella pneumoniae* than *Salmonella typhi*, especially with the methanolic extracts. This reflects its potency over the hot aqueous extract. Achinto and Munirudin (2009), reported that a low MIC value of medicinal plant extract indicated a better antibacterial activity. This according to Ogodo *et al*. (2017), is a reflection of strong antibacterial and antimycotic effects on the test organisms, particularly the with organic extractant. The better minimum bactericidal effects (MBC) exhibited by methanolic extractant over aqueous agreed with Nas *et al* (2017), who posited that organic solvents generally have better solubility than aqueous, hence higher concentration of bioactive constituents and better suppressive and lethal effects.

The ratio of MBC/MIC otherwise known as antibiotic power (AP) obtained, showed that methanolic extracts of G. kola(GMetOH) and combined extract (A+GMetOH) gave bacteriostatic effects on *K. pnemoniae* and *S. typhi* respectively. While all other extracts gave bactericidal effects on the test organisms. This corroborated the findings of Noumedem *et al.* (2013), who stipulated that a sample is bactericidal when the ratio MBC/MIC ≤ 4 and bacteriostatic when this ratio is >4.

The fractional inhibitory concentration indices (FICI) obtained reflect the effects of combined plant extracts on the test microbes. FICI is synergistic, if value is ≤ 0.5, additive (0.5–1.0), indifferent (1–4.0) or antagonistic (≥ 4.0) (Schelz *et al*., 2006; Iten, 2009). Consequently, the effects of combined extracts on *S. typhi*, was indifferent. However, the effects of the combined extracts on *K. pnemoniae* were indifferent and antagonistic for A+GH_2O and A+GMetOH respectively. Ncube *et al* (2012), had reported antagonistic interaction effect of *Tulbaghia violacea* leaf samples against *Bacillus subtilis, Escherichia coli* and *K. pneumonia*. According to Olayinka *et al* (2009), combining plant extracts against disease causing microbes provide an alternative approach to tackle infections caused by multi-resistant pathogens (Van Vuuren and Viljoen, 2011). These results also confirmed findings of (Shaza *et al*., 2014; Mahajan *et al*., 2014).

Conclusion and Recommendations

The study revealed the seed extracts of the test plants (*Aframomum melegueta*, *Garcinia kola* and the combined extracts) with bioactive substances whose presence was dependent on the extractants (methanolic and aqueous solvents). Better inhibition was recorded with methanolic extract against *Klebsiella pneumoniae* than the *Salmonella typhi*, reflecting their relative sensitivity. All extracts indicated bactericidal, while GMetOH and A+GMetOH gave bacteriostatic effects against *K. pneumoniae* and *S. typhi* respectively. The combined extracts showed some fractional inhibitory effects against the test organisms.

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