

Original Research Article

***ANTIMICROBIAL EFFECTS OF AQUEOUS EXTRACT OF
GARCINIA KOLA NUTS ON SALMONELLA ISOLATES FROM
CHICKEN DROPPINGS***

Abstract

Aim : The onerous threat of antimicrobial resistance to public health highlights the need for continuous research that will discover more potential medicinal plants that possess inhibitory potentials on bacteria especially those with multidrug-resistant qualities. Hence, this study investigated the antibacterial effect of hot and cold aqueous extracts of *Garcinia Kola* on *Salmonella* isolates.

Study Design: This is experimental research involving fecal sample of chicken.

Place and Duration of Study: This research was carried out in Wukari metropolis.

Study Design: Sterile universal containers were used to collect one gram each of chicken fecal samples from domestic chicken coops and dissolved in Buffered Peptone Water to recover injured cell. A loop-full of the sample was streaked on prepared *Salmonella-Shigella* Agar plates. Suspected isolates were confirmed molecularly using PCR to identify the *invA* virulent gene from the isolate. Hot and cold water served as a menstruum for extracting bioactive contents from *Garcinia Kola*. Following evaporation of the crude hot and cold-water extracts, 0.25mg, 0.5mg, 1.0mg, and 2.0mg of the extract were respectively dissolved into 10 ml of distilled water. The Agar-well diffusion method was used in conducting the antimicrobial susceptibility tests.

Results: Significant zone of inhibition (ZIB) of 9mm, 12mm and 17mm was observed for isolates subjected to 0.50mg-10 ml, 1.00mg-10 ml, and 2.00mg-10 ml concentrations of cold-water extracts respectively while No zone of Inhibition (NZI) was observed at 0.25g-10 ml concentrations. For hot water extracts, growth inhibition ranging from 9mm, 11mm, 15mm, and 23mm was respectively noticed for concentrates of 0.25mg-10 ml, 0.50mg-10, 1.00mg-10 ml, and 2.00mg-10 ml. Heavy growth persisted for the negative control plate which contained distilled water without extracts. Augmentin 30µg was used as a positive control (≥31mm).

Results: This research simply has amplified the medicinal importance of the consumption of *Garcinia Kola* particularly as it relates to the management of gastroenteritis caused by Zoonotic Non-enteric *Salmonella*. However, the clinical toxicity and safety of the plant need more understanding.

Keywords: *Antimicrobial resistance, Aqueous, Chicken, Salmonella, Garcinia Kola*

1. Introduction

Traditional use of plant with medicinal potentials has advanced the discovery of novel antimicrobials [1]. Before the 20th century, not less than 79% of medications were produced from phytochemicals thereby stimulating the prominence of pharmaceutical market [2]. More recently, the use of medicinal plant has been on the increase and it is not unconnected to their established cost effectiveness, and safe qualities with considerable side effects [3].

Salmonella specie is a major food-borne pathogen that has been repeatedly linked to poultry birds [4]. *Salmonella* is a non-spore forming, Gram-negative rods [5]. *Salmonella enterica* is the causative agent of acute gastroenteritis and enteric fever [6]. Worldwide, not fewer than 1 million reported clinical cases of food-borne gastroenteritis are caused by *Salmonella* resulting to over 100,000 deaths [7]. Food product of animal origin, such as chicken and other poultry products are more prominent in harbouring the pathogen from where it is easily transmitted to humans [7]. *Salmonella* is susceptible to some antibiotics such as fluoroquinolones. However, occurrences of *Salmonella* resistivity to conventional drugs of choice has been observed [8].

The intensifying pattern of drug resistance demands a total attention. [9,10]. [8] has opined that the multidrug resistivity by *Salmonella* is of huge negative impact to public health management. Evidence of multidrug resistance by *Salmonella* to ampicillin, chloramphenicol, gentamycin and tetracycline has been documented [8,11]. Traditional medicine has shown complementary efforts to allopathic medicine toward achieving health and wellbeing [12]. This has necessitated the need to screen antimicrobials from plant origin in the management of health and wellbeing [6].

Garcinia Kola which is also known as bitter kola has gained therapeutic prominence over the years as it is used basic illnesses [13]. *Garcinia Kola* belongs to the genus *Garcinia*, and a member of the *Guttiferae* family [14]. It is found in many parts of tropical and sub-tropical Africa lowland forest as its natural habitat. *Garcinia Kola* nuts are smooth oval shaped, with brown nut coat. The nuts and bark of *Garcinia Kola* which is characterized by a bitter taste earning it the name "Bitter kola" have been in used from even the medieval era as remedy for common cold and fever [15]. *Garcinia Kola* is considered an economic plant across West African countries and beyond as its trade is still viable to the indigenous communities and villages in most part of Africa [13]. *Garcinia Kola* is willingly offered to visitors, especially in southern Nigeria, at home functions or ceremonies [15]. There are evidences that the nuts are used in formulating several herbal remedies. Its therapeutic relevance in managing cases of malaria, liver problems, bronchitis, throat contaminations, colic, feverish conditions and hypertension has been attributed to its phytochemical contents such as alkaloids, saponins, tannins, glycosides, cardiac, anthraquinones and flavonoids [16]. These phytochemicals contained in every part of *Garcinia Kola* coupled with its effectiveness as remedies has earned it the reputation as a "wonder plant" [13]. Nuts of *Garcinia Kola* are chewed to produce an aphrodisiac and therapeutic effect particularly for treating gonorrhoea [15].

The foreseen relevance of traditional medicine advancement in tackling antimicrobial resistance has instigated the need for in-vitro investigation of the microbial inhibitory effects of extracts of *Garcinia Kola* against *Salmonella* isolated from the chicken source. Hence, this study aims to examine the antimicrobial effect of aqueous extract of *Garcinia Kola* nuts on *Salmonella* specie.

2. Material and Method

2.1 Study area: Upon ethical approval from the Department of Microbiology, Federal University Wukari, this research was conducted in Wukari Local Government Area of Southern Taraba state, and the major ethnic group in the town is the Jukun people. Wukari is home to federal University Wukai and Kwararafa University.

2.2. Collection and Identification of *Garcinia Kola* nuts: *Garcinia Kola* nuts were bought in the open market. Upon identification by a plant expert, the plant was immediately taken to the laboratory for further analysis using standard microbiological techniques and procedures.

2.3. Preparation of hot and cold aqueous extract of the *Garcinia Kola* nuts: The *Garcinia Kola* nuts were immediately grinded and were weighed. Various concentration of the *Garcinia Kola* extracts was made by weighing specified grams of the grinded *Garcinia Kola* and macerating it into 10ml of both hot and cold water respectively and were stored in well corked sterile universal bottles for 3 days. Thereafter, the extract was parted from the residues by filtering using the filter paper and funnel into a sterile universal bottle giving a final concentration of 0.25mg-10ml, 0.5mg-10ml, 1mg-10ml and 2mg-10ml.

2.4. Collection of poultry droppings: Sterile spatulas were used to collect 1g of dried and fresh fecal samples each from various poultry units in Wukari. The droppings were collected in sterile sampling containers, labelled respectively and immediately transported to the laboratory Federal University Wukari where they were processed using standard microbiological techniques.

2.5. Culturing of the poultry droppings (dried and wet) on *Salmonella Shigella* agar: 1g of both dried and wet Chicken fecal sample was collected and in a sterile container containing in 10ml of sterile water. Pour plate technique was used to culture samples in already prepared

Salmonella-Shigella Agar (SSA) media and rocked gently to obtain a homogenous mix and allowed to cool to 50°C. The inverted plates were incubated at 37°C overnight. Noticeable colonies of presumed *Salmonella* isolates with greyish white, low convex, smooth, and translucent appearance were sub cultured into Nutrient agar and placed in an incubator for 24 hours at 37°C to obtain pure culture. Isolates were further subjected to biochemical analysis and Gram staining.

2.6. Molecular confirmation of Isolates: Polymerase Chain Reaction was used to detect the *invA* gene which is considered a biomarker for the molecular confirmation of *Salmonella* isolates. The DNA of culturally and biochemical confirmed *Salmonella* isolates was extracted by boiling method. The PCR procedure was carried out using a reaction combination of reagents consisting sterile distilled water, GoTaq PCR reaction buffer, GoTaq DNA polymerase, 1 µl DNA template, 0.2 mM PCR nucleotide mix, and 0.5 µl each of forward and reverse DNA primers giving a final volume of 50 µl. Specific primers selected to target 284 bp segment of the *invA* gene of *Salmonella* are shown in Table 2. The PCR reagent mixture was placed in a thermocycler with an initial denaturation at 94°C for 10 min, followed by another denaturation consisting of 35 cycles at 94°C for 1 min, then annealing at 58.3°C for 1 min, and 72°C elongation for 1 min followed by a final extension for 10 mins at 72°C. A volume of 6 µl mixture of amplicon and loading solution were subjected to electrophoresis for 45 minutes at a constant voltage of 100 v. to confirm amplicon sizes using 2% agarose gel prepared with tris-borate-ethylenediaminetetraaceticacid (TBE) and stained with 5 µg/ml ethidium bromide. DNA band patterns were compared against a 100 bp marker to determine the amplified product size.

2.7. Antimicrobial effects of *Garcinia Kola* extract on isolate: Using the spread plate method, 1ml of the suspension of the test organism was introduced into each plate of already prepared Nutrient agar. Sterile cork borer was used to make 7mm wells for each concentrates of hot and cold extracts. Positive control well contained Augmentin 30µg while negative control well did not contain any extract concentrates but distilled water. Then the plates were put way for 48 hours in an incubator at 37°C.

3. Results

Cold and hot water extracts of *Garcinia Kola* nuts demonstrated enormous antimicrobial potential when screened for their bactericidal effects against foodborne pathogens isolated from domestic chicken coops droppings.

Table 1: Biochemical and morphological identification of isolates

Isolate	Biochemical test						Gram stain
	CAT	INDO	MR	LAC	SUC	GLU	
<i>Salmonella</i>	+	-	+	-	-	+	<i>Negative bacilli</i>

The biochemical and morphological characteristics of presumed *Salmonella* are presented in Table 1, while Table 3 represents the growth inhibition pattern of the respective concentrations of hot and cold-water extracts of *Garcinia Kola* nuts on *Salmonella* isolates. A significant zone of growth inhibition of 9 mm, and 12 mm 17 was observed for isolates subjected to 0.5mg-10 ml,

1.0mg-10 ml, and 2.0mg-10 ml concentrations of cold-water extracts of *Garcinia Kola* nuts respectively while No zone of Inhibition (NZI) was significant at 0.25mg-10 ml concentrations. For hot water extracts of *Garcinia Kola* nuts, growth inhibition ranging from 9 mm, 11 mm, 15 mm and 23 mm was detected for concentrates of 0.25mg-10 ml, 0.5mg-10, and 1.0mg-10 ml and 2.0mg-10 ml respectively. Heavy growth persisted for the negative control plate which contained distilled water without extracts. Augmentin 30µg was used as a positive control with zone of inhibition of 31mm.

Table 2: Primer sequences for *invA* gene

SN	Target gene	Primer sequence	Amplicon size	Reference
1	<i>invA</i>	GTG AAA TTA TCG CCA CGT TCG GGC AA TCA TCG CAC CGT CAA AGG AAC C”	284bp	[17]



Figure 1 Agarose gel electrophoresis showing PCR amplification products of 284 bp *invA* gene in *Salmonella* isolates

Lane M, 100 bp marker; Lane 1, test samples (*Salmonella*); Lane N, Negative control

Table 3: Antimicrobial effects of *Garcinia Kola* nut extracts on *Salmonella* isolate

Concentration	<i>Garcinia Kola</i>		Positive Control	Negative Control
	Cold water extracts	Hot water extracts	Augmentin 30µg	Distilled water
0.25 mg-10ml	NZI	9 mm	31mm	NZI
0.5 mg-10ml	9 mm	11 mm		
1.0 mg-10ml	12 mm	15 mm		
2.0 mg-10ml	17 mm	23 mm		

Key: ≤ 14mm, (R)Resistant; ≥ 20mm, (S)Sensitive; 15mm-19mm, (I)Intermediate; NZI, NO Zone of Inhibition.

4. Discussion

The antimicrobial analysis of *Garcinia Kola* nuts extracts from this study has unearthed its effectiveness in inhibiting the growth of zoonotic *Salmonella* isolates from chicken fecal samples and has highlighted the need to further investigate the effects on other pathogenic organisms. Similarly, the extract of *Garcinia Kola* nut using methanol has shown to inhibit the growth of *Shigella*, *Klebsiella Pneumoniae* and *Escherichia coli* isolates [14]. This antimicrobial effect is not unconnected to the vital phytochemical contents of *Garcinia Kola* which are not limited to alkanes, fatty acids, flavonoids, alkaloids, and amines [13]. Phytochemicals that customarily provide protection to plants from harsh ecological circumstances also have bactericidal abilities with the potential for use in healing diseases [18]. The use of plants with antimicrobial potential has increased tremendously recently. Traditional medicines are now being prioritized over allopathic medicine in achieving universal health coverage for their cheap and ready availability, while the efforts of synthetic antibiotics use as remedies are limited successfully by multidrug resistance [19]. The possibilities of microorganisms inflicting resistant genes and mechanisms on antimicrobials of plant origins are slim because of their sophisticated molecular features [20]. Plant with antimicrobial potentials synthesizes secondary metabolites that provide protection against microbial resistance mechanism by infringing on intermediary metabolism and apoptosis [21]. Also, plants can obstruct cellular “protein to protein” interfaces, thus activating effective modulators of signal transduction and immune response [22]. Nonetheless, there is a likelihood of bacteria to assume resistance to antimicrobials of plant origins. This opinion has been aggressively disputed by [23] which recommended more research to substantiate the claim. Presumed resistance by microorganisms to antimicrobials of plant extracts may be due to unregulated activities of quacks and unskilled professionals who do not strictly follow the standard rudiments of phytochemical extracting processes as well as poor choices in identifying potential plants of medicinal value.

This current study among its objectives was able to substantiate the disparity in the efficacy of cold and hot aqueous extracts. Contrary to the findings of [1], that ethanol is more effective than any other menstruum in extracting bioactive contents of plants, this present research has shown that hot water is equally efficient in phytochemical extraction. This finding is consistent with that of [24] which observed that water at a high temperature extracted more than methanol yields of bioactive contents from plant. The choice of the aqueous extraction technique used in this study is necessitated by its harmless and inexpensive potentials in pursuant to the core policy of Universal health coverage which warrants that all individuals not minding their financial and social status can get quality healthcare services. Owing to its availability qualities, water is the more cost-efficient solvent used by traditional healers in the preparation of medicines from varied spectrum of potential medicinal sources including plants [25]. However, the use of water is disadvantaged by its natural microbial contamination. If opportunities for TM are maximized, there will be better and more affordable health coverage in Nigeria and other developing Nations. In India, over 90% of individuals depend on remedy from plant extraction for their basic well-being [25, 26]. In Nigeria, tradomedical practitioners are more readily available than allopathic doctors to the population [19]. This portrays that access to tradomedical practitioners is more practicable compared to conventional medicine. This goes a long way to complement other reasons why people particularly in Africa has more willingness to patronize the former than the latter owing to the socioeconomic impediments in most part of Africa.

5. Conclusion

The potential for managing zoonotic gastroenteric infections caused by *Salmonella* with antimicrobials of plant origin such as *Garcinia Kola* is possible. It is not only cost-effective but, reduces the possibility of antimicrobial resistance. The universal health coverage policy in

Nigeria is achievable, only when opportunities for traditional medicine are exploited to complement the underwhelming efforts of orthodox medicine.

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