

Antisalmonella Potency of Varying Extracts of *Garcinia kola*

Abstract

Plants are a source of first-line treatment for most humans, particularly in Africa and offer nutrition for most terrestrial ecosystems. The world's attention has been called repeatedly to the therapeutic marvels of plants, one of which is *Garcinia kola*. This study aims to determine the antisalmonella efficacy of several *Garcinia kola* leave and seed extracts. Extracts of leaves, seeds, and seed/leaf were obtained using methanol and ethanol as extraction solvents. Inoculated *Salmonella typhi* were observed for 24 hours using the well diffusion method to determine zones of inhibition. All extracts were found to be effective against *Salmonella typhi*. 100/50 mg/ml methanol leave extract produced a 14/12mm zone of inhibition, whereas 100/50 mg/ml methanol seed extract produced a 13/11mm zone of inhibition. 100/50 mg/ml ethanol leave extract showed a 17/10mm inhibition zone, while 100/50 mg/ml ethanol seed extract showed a 16/14mm inhibition zone. The inhibition zone of methanol seed/leaf extract of 100/50 mg/ml was 17/12mm, while the inhibition zone of ethanol seed/leaf extract was 19/14mm. While all concentrations of plant extracts were effective against *Salmonella typhi*, greater concentrations created larger zones of inhibition and the plant extracts outperformed the control. Seed/leaf extracts outperformed seed extract and leave extract in most cases. The results showed that ethanol was a better extraction liquid and that the leaf extracts were more potent; however, this is not true in all cases. To stimulate local mitigation of illnesses caused by *Salmonella typhi*, more research on the antisalmonella effectiveness of *Garcinia kola* seed and leave extracts should be done.

Keywords: Antisalmonella, *Garcinia kola*, Potency, Extracts, *Salmonella typhi*.

1. Introduction

Plants are not just essential for human life in terms of food and health but also by the provision of oxygen required for the maintenance of human life [1]. Phototherapy which seeks to prevent or cure diseases with natural medications prepared by using all or some parts of plants is one of the oldest natural methods of therapy known in human history [2]. In many parts of the world, medicinal plants have for thousands of years been used as traditional treatments for numerous human diseases and in many rural areas of the developing countries, they continue to be used as the primary source of medicine [3]. The World Health Organization (WHO) has reported that about 4 billion humans around the world try to cure their health problems with herbal remedies at the first step [4]. In developing countries, about 80% of people use traditional medicines for their health care while in affluent countries, herbal active ingredients account for around a quarter of all prescription medications [5,6]. Thousands of phytochemicals derived from plants are safe and effective, with little side effects having anticancer, antibacterial, antioxidant, antidiarrheal, analgesic and wound healing properties for which plants are responsible for around a quarter of all pharmaceuticals used in modern medicine [7,8]. There are a variety of reasons for the increase in plant research: reduction in antibiotics effect due to microbial resistance, the desire of developing countries without a competent chemical industry to gain an easy and cost-effective treatment option, harmful side effects of synthetic compounds used for therapeutic purposes, multiple drugs effect and the fact that some substances obtained from herbal drugs can

be extracted more economically; these reasons have sparked increased interest in the identification of novel anti-infective compounds in the management of diseases caused by microbes [9,10]. One plant that has been frequently used for its nutritional and medicinal virtues is *Garcinia kola*.

Garcinia kola is a dicotyledonous plant that belongs to the clusiaceae or guttiferaceae family [11]. This plant has been recognized as an indigenous medicinal plant found in the tropical rainforest of Benin, Sierra Leone, Democratic Republic of Congo, Ivory Coast, Gabon, Ghana, Liberia, Nigeria, Senegal and Cameroon, where it is one of the most valued trees [12]. *Garcinia kola* is a tree that is well branched and evergreen that grows to a height of about 12 meters; it has large opposing leaves (12" long by 7" broad) with short petioles that grow near the base of the branches, but the leaves at the end of branches are considerably smaller (5" by 2"). The leaves are oval in shape, somewhat dilated at the base and full green on top with a greenish underside; the tree has a straight and cylindrical trunk with smooth bark that is dark-brown outside and pinkish inside. It has a dense crown with erect dropping branches [12,13]. The tree produces reddish golden or orange coloured fruit categorized as a berry, that has a rugose epiderm coated totally in scratchy hairs and the size is compared to that of an apple. Each fruit contains two to four yellow seeds and a sour tasting pulp [14].

Garcinia kola is one of the medicinal plants which has been used in African ethnomedicine because it has purgative, antiparasitic and antimicrobial properties [15]. *G. kola* produces a wide range of products, including fruits, seeds, bark, twigs, leaves, and wood, but the seeds are the most important. Both the seeds and the bark are used in folk medicine to treat stomach and liver problems. Headaches, laryngitis, bronchitis, malaria, and gonorrhoea can all be relieved by chewing the seeds [16,17]. In West Africa, the plant's root is used as a bitter chewing stick, whereas in southern Nigeria, the stem is used as a chewing stick. [18,19,20]. The nuts are chewed traditionally as a masticatory substance to stimulate the flow of saliva; the nuts are also chewed for aphrodisiac purposes or used in herbal medicine to treat coughs, diarrhoea, and chest colds. [21,22]. The seeds of *Garcinia kola* are culturally and socially significant in some parts of West Africa, where they are used for traditional hospitality at private, social, and cultural activities [16]. The barks of the nuts are widely traded and consumed as a stimulant. [23,21]. It has been found that the seeds have broad spectrum antibacterial action [16]. Leaf and seed extracts have shown varying antibacterial action on both gram-negative and gram-positive bacteria.

Salmonella typhi is a Gram-negative, obligate, facultatively anaerobic, short, rod-shaped bacterium which can grow at 5-45°C that belongs to the serogroup D within subspecies I of the genus *Salmonella* and is represented by the antigenic formula 9,12:d:- [24,25]. It is a serovar of *Salmonella enterica* which lives only in humans and causes typhoid fever which has long been a problem in developing countries [26,27]. Typhoid fever is more common in children and young people and it is linked to low-income communities with a lack of sanitation [28]. In 2001, typhoid fever caused 21.7 million illnesses and an estimated 216,000 people died of it. In 2010, the International Vaccine Institute estimated that 11.9 million people in low- and middle-income countries got typhoid fever, with 129,000 people dying as a result of the disease [29,30]. According to the World Health Organization (WHO), the global typhoid fever disease burden is

estimated to be 11-20 million cases annually, with 128 000–161 000 deaths per year [31]. In the pre-antibiotic era, mortality rates were as high as 15% or more but with the discovery of antibiotics, death rates have dropped to fewer than 1% [32]. For many years, two vaccines have been used to protect people from typhoid disease. These vaccines do not give long-term protection and are not recommended for children under the age of two. In December 2017, WHO prequalified a novel typhoid conjugate vaccine with longer-lasting protection for use in infants as young as six months [31]. *Salmonella typhi* has however developed antimicrobial resistance, initially to the first-line antibiotics chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole [33]. To this end plant substitutes, like bitter kola, have been widely encouraged for alternative use.

Bitter kola's seed and leaf contain a complex blend of biflavonoids and prenylated benzophenones; tannins, cardiac glycosides, saponins, alkaloids, hydroxymethyl anthraquinone, phlobatannins, polyphenols, glucosides, garcinnoic acid, and reducing compounds found in *G. kola* have anti-inflammatory, anti-diabetic, and anti-hepatotoxic properties. [34,35,36]. *Garcinia kola* has antibacterial characteristics and is beneficial in the treatment of infectious disease, according to clinical data, while also minimizing many of the negative effects commonly associated with synthetic antimicrobials [15]. Ethanol, cold water and hot water extracts of *G. kola* inhibited *S. aureus* and *E. coli* according to Okigbo and Mmeko 2008. *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumonia* were all inhibited by the methanolic leaf extract of *Garcinia kola*, while *Staphylococcus aureus* and *Escherichia coli* were inhibited by the aqueous leaf extract but both methanolic and aqueous extracts had no antifungal efficacy against *Candida albicans*; the lowest inhibitory concentration of *Garcinia kola* leaf extracts ranged from 25 to 50 mgL⁻¹ [38]. The ethanolic and aqueous extracts of *Garcinia kola* seed when used against selected test organisms *Streptococcus pneumonia*, *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Escherichia coli* showed that the seed extracts possess reasonable antibacterial activity but to varied zones of inhibition [39]. To Indabawa and Arza 2011, *Salmonella typhi* was completely resistant to alcohol extracts of *Garcinia kola* but hot water extract was active against it. Seed and leaf extracts using acetic acid inhibited clinical isolates of *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, and *Streptococcus pyogenes* with zones of inhibition of 44mm and 37mm, respectively [41]. As the drive of most Africans is in the direction of easily accessible and cost-effective treatment options; this study seeks to establish the antisalmonella potency of varying extracts of *Garcinia kola* leaves and seed.

2. Method

Sample Collection

Garcinia kola seeds were purchased from dealers of bitter kola seeds in Bori market of Khana LGA. The leaves were gotten from a tree in Yehge village in Gokana LGA. All collected plant samples were transported in sterile polythene bags to the Microbiology Laboratory of Kenule Beeson Saro-Wiwa Polytechnic Bori in Khana LGA.

Sample Preparation

Leaves of *G. kola* were washed under running tap; they were then oven dried at 80°C for 48 hours. The leaves were ground in a clean blender and the ground leaves were stored in a sterile

container until time for use. The seeds were washed under a running tap, dehusked and washed again. They were then ground in a clean blender and the ground seeds were immediately used in extraction.

Extraction

1. Twenty grams of dried, ground leaves of *G. kola* were soaked in 200ml of ethanol (99.7%) for three days.
2. Twenty grams of ground seeds of *G. kola* were soaked in 200ml of ethanol for three days.
3. Ten grams of dried ground leaves and 10g of ground seeds of *G. kola* were mixed; this mixture was then soaked in 200ml of ethanol for three days.
4. The procedure for 1-3 was done for methanol (99.7%) as the extraction liquid.

After three days, the mixtures were filtered using new sterile handkerchiefs, the filtrates were evaporated to dryness using a water bath and stored in the refrigerator at 4°C until time for use [37,40].

For each extract, two concentrations were prepared: 100mg/ml and 50mg/ml using the formula $C_1V_1=C_2V_2$. The extracts were then reconstituted using Dimethylsulfoxide (CH₃)₂SO M=78.14.

Test Organism

Pure isolates of *Salmonella typhi* were obtained from stock culture at Microbiology Laboratory of Kenule Beeson Saro-Wiwa Polytechnic and were maintained on fresh nutrient agar slants (in a bijoux bottle and kept in refrigerator at 4°C). A suspension of *S. typhi* was prepared that matched the turbidity of 0.5 McFarland standard.

Culture Media

Salmonella-Shigella (SSA), Nutrient Agar (NA) and Muller Hinton Agar (MHA).

Determination of Antisalmonella Activity

Mueller Hinton's Agar was prepared according to manufacturer's standard by dissolving 7.6g of the agar powder in 200ml of distilled water and preheating to mix properly in sterile conical flasks (serially). Each flask was stopped with a non-absorbent cotton wool and the medium was sterilized by autoclaving at 120°C for 15 minutes at 15Psi. After sterilization, the media was allowed to cool and was dispensed into sterilized petri dishes (18) and allowed to set. Well diffusion method was used to test for antisalmonella activity [41]. Before the transfer of organisms from the bacterial suspension, the surfaces of the agar in the petri dishes were dried in a hot air oven for 5 minutes at 60°C. Organisms from suspension were seeded onto freshly dried agar and distributed evenly by means of sterile swab stick. With sterile cork borer, wells were made in the agar medium and labelled appropriately (12 for plant extracts and 6 for tetracycline as control). The wells were filled with reconstituted plant extracts and tetracycline (200mg/ml) and allowed to stay for a while for the extracts to diffuse into the agar. Plates were incubated in an incubator for 24hrs.

Measurement of Zone of Inhibition

Clear zone of inhibition around the well that indicates the antibacterial potency of plant extracts and tetracycline against the test organism was measured using a transparent meter rule and the measurements were recorded in millimeter (mm).

3. Result and Discussion

The results of the antibacterial or antisalmonella activity of varying extracts of *Garcinia kola* seed, leaves and seed/leave on *Salmonella typhi* expressed in zones of inhibition is presented in tables 1-6.

Table 1: Antibacterial Activity of *Garcinia kola* (Methanol) Leave Extract

Test organism	Concentration (mg/ml)	Zone of Inhibition (mm)	Control (Tetracycline 200mg/ml) (mm)
<i>Salmonella typhi</i>	100	14	28
	50	12	

Table 2: Antibacterial Activity of *Garcinia kola* (Methanol) Seed Extract

Test organism	Concentration (mg/ml)	Zone of Inhibition (mm)	Control (Tetracycline 200mg/ml) (mm)
<i>Salmonella typhi</i>	100	13	28
	50	11	

Table 3: Antibacterial Activity of *Garcinia kola* (Ethanol) Leave Extract

Test organism	Concentration (mg/ml)	Zone of Inhibition (mm)	Control (Tetracycline 200mg/ml) (mm)
<i>Salmonella typhi</i>	100	17	28
	50	10	

Table 4: Antibacterial Activity of *Garcinia kola* (Ethanol) Seed Extract

Test organism	Concentration (mg/ml)	Zone of Inhibition (mm)	Control (Tetracycline 200mg/ml) (mm)
<i>Salmonella typhi</i>	100	16	28
	50	14	

Table 5: Antibacterial Activity of *Garcinia kola* (Methanol) Seed & Leave Extract

Test organism	Concentration (mg/ml)	Zone of Inhibition (mm)	Control (Tetracycline 200mg/ml) (mm)
<i>Salmonella typhi</i>	100	17	28
	50	12	

Table 6: Antibacterial Activity of *Garcinia kola* (Ethanol) Seed & Leave Extract

Test organism	Concentration (mg/ml)	Zone of Inhibition (mm)	Control (Tetracycline 200mg/ml) (mm)
<i>Salmonella typhi</i>	100	19	28
	50	14	

100mg/ml

The ethanol leave extract had a higher zone of inhibition (17mm) as compared to methanol leave extract (14mm).

The ethanol seed extract also had a higher zone of inhibition (16mm) as compared to the methanol seed extract (13mm).

The leave and seed extract of ethanol also had a higher zone of inhibition (19mm) when compared with that of the methanol extract (17mm).

The seed and leave (SL) extract of methanol had a higher zone of inhibition (17mm) as compared to the leave (L) extract (14mm) and the seed (S) extract (13mm). The relationship can be presented as SL (17mm) > L (14mm) > S (13mm).

For ethanol extracts, the same relation was seen SL (19mm) > L (17mm) > S (16mm).

In all cases of the 100mg/ml, the plant extracts did better than the control.

50mg/ml

The methanol leave extract had a higher zone of inhibition (12mm) as compared to ethanol leave extract (10mm).

The ethanol seed extract had a higher zone of inhibition (14mm) as compared to the methanol seed extract (11mm).

The leave and seed extract of ethanol also had a higher zone of inhibition (14mm) when compared with that of the methanol extract (12mm).

The seed and leave (SL) extract of methanol had the same zone of inhibition (12mm) as compared to the leave (L) extract (12mm) but higher than the seed (S) extract (11mm). The relationship can be presented as SL (12mm) = L (12mm) > S (11mm).

For ethanol extracts, the relation seen was SL (14mm) = S (14mm) > L (10mm)

In all cases of the 50mg/ml, the plant extracts also did better than the control.

There are few general statements that are to be drawn from the results; higher concentration of the plant extracts produced higher zones of inhibition, all concentrations of the plant extracts showed potency against *Salmonella typhi* and the plant extracts did better than the control. Seed and leave extracts generally performed better than seed extract and leave extract although 50mg/ml ethanol seed and leave extract had the same zone of inhibition as seed extract (all 14mm). The results showed ethanol as a better extraction liquid and the leave extracts as having greater potency; but this is not general.

Plants are essential for human life [1]; having been used to treat numerous human diseases [3] with phytochemicals from plants having antibacterial properties [7,8] and that *Garcinia kola* has antibacterial characteristics [15] have been buttressed by the findings of this work. *G. kola* produces a wide range of products including fruits, seeds, twigs, leaves and wood [16,17]; the results of this work have shown antibacterial significance of the leaves and seeds. Bitter kola seeds and leaves contain a complex blend of bioflavonoids [34,35,36] with the seed having a broad spectrum antibacterial action [16]; the findings of the work has shown the antibacterial action of bitter kola seeds and it's known that these actions are due to the phytochemicals. Afolabi et al., 2020 showed that ethanol extracts of *Garcinia kola* seeds when used on *Salmonella typhi* showed reasonable antibacterial activity but to varied zones of inhibition; the findings of this work is in accord. Seed and leave extracts using acetic acid inhibited *S. typhi* [41]; methanol and ethanol extracts here inhibited the same organism. According to Indabawa and Arzai 2011, *Salmonella typhi* was completely resistant to alcohol extracts of *Garcinia kola* but the findings of this work showed that methanol and ethanol extracts of the seed and leave extracts inhibited *Salmonella typhi* at different concentrations.

4. Conclusion

Methanol and ethanol extracts of *Garcinia kola* seed, leave and seed/leave showed potency against *Salmonella typhi*. Methanol leave extract of 100mg/ml produced 14mm zone of inhibition while 50mg/ml methanol leave extract produced 12mm zone of inhibition. Methanol seed extract of 100/50 mg/ml showed 13mm/11mm zone of inhibition. Ethanol leave extract of 100/50 mg/ml showed 17mm/10mm zone of inhibition while ethanol seed extract of 100/50 mg/ml showed 16/14mm zone of inhibition. Methanol seed/leave extract of 100/50 mg/ml showed 17/12mm zone of inhibition while ethanol seed/leave extract produced 19/14mm zone of inhibition. Whereas all concentrations of the plant extracts showed potency against *Salmonella typhi*, higher concentrations of the plant extracts produced higher zones of inhibition and the plant extracts did better than the control. Seed/leave extracts generally performed better than seed extract and leave extract. The results showed ethanol as a better extraction liquid and the leave extracts as having greater potency; but this is not general.

There has been a lot of study on the seed of *Garcinia kola* and this should continue particularly the curative aspect. Studies on the leave potential should also be rapidly intensified. Further studies on the antisalmonella potency of *Garcinia kola* seed and leave extracts be conducted to encourage local mitigation of ailments due to *Salmonella typhi*.

NOTE:

The study highlights the efficacy of "herbal medicine" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

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