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## Antimicrobial Efficacy of a Syrup Formulated from Methanol Extract of *Garcinia Kola* Seed

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### ABSTRACT

This study was designed to evaluate the *in vitro* antimicrobial properties of the methanol extract of *Garcinia kola* seed and its syrup formulation and compare the latter with commercial antibiotic syrups against *Escherichia coli* and *Staphylococcus aureus* by the standard agar diffusion method. The physicochemical properties of the syrups were also assessed. The test micro-organisms were significantly susceptible ( $p < 0.01$ ) to the extract (MIC =  $3.50 \pm 0.04$  mg/ml for *Escherichia coli* and  $5.05 \pm 0.12$  mg/ml for *Staphylococcus aureus*) and the syrup formulation (IZD =  $12.00 \pm 0.05$  mm for *Escherichia coli* and  $24.00 \pm 0.19$  mm for *Staphylococcus aureus*). The antibacterial activity of *Garcinia kola* syrup was greater than amoxicillin and metronidazole syrups but less than co-trimoxazole syrup against the test micro-organisms. The physicochemical data revealed that the pH of *Garcinia kola* syrup compared favourably with the pH of the commercial syrups, whereas the viscosity of the herbal syrup was less than those of commercial syrups thus enhancing its administration. This research has shown that methanol extract of *Garcinia kola* seed can successfully be formulated into syrup. Standardization of the *Garcinia kola* syrup will hopefully expose new frontiers by improving on the available safe, stable and efficacious dosage forms of this herbal medicine.

**Keywords:** *Garcinia kola* seed, syrup, antibacterial properties, formulation.

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### INTRODUCTION

The use of plant materials as spices, condiments and for medicinal purposes dates back to the history of mankind [1]. Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids, which have been found *in vitro* to have antimicrobial properties [2]. A number of phytotherapy manuals have mentioned various medicinal plants for treating infectious diseases due to their availability, fewer side effects and reduced toxicity [3]. *Garcinia kola* Heckel (Guttiferae), a plant of West and Central African origin [4], is commonly referred to as bitter kola for its bitter taste and has the popular acronym "wonder plant" amongst the

Southwestern Nigerian people because every part of it has been found to be of medicinal importance [5]. The medicinal uses of *Garcinia kola* as reported in the literature include its use as anti-parasitic, antimicrobial, antiviral, anti-inflammatory, purgative, antidote to the effects of *Strophantusgratus*, remedy for guinea-worm infection and for the treatment of gastroenteritis, rheumatism, asthma, menstrual cramps, bronchitis, throat infections, colds, cough, and liver disorders [6, 7]. The plant is also used as antidiabetic on account of its bitter principle, antioxidant, and for chemoprevention of aflatoxin B1 and for its antihepatotoxic activities [8 – 12]. The



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seed has proven antimicrobial activities [13 – 17] and it has been employed in the treatment of various ailments. The phytochemicals obtained from *G. kola* as documented in literature include biflavonoids, xanthenes, kolanone, amekoflavone, 24-methylenecyclartenol, coumarine and prenylatebenzophenones[18]. These phytochemical compounds are known to play important roles in the bioactivity of medicinal plants.

The formulation of plant drugs into modern pharmaceutical dosage forms might build into them many excellent physical and chemical properties such as dosage precision, a prolonged shelf life, ease of administration, and a greater acceptance due to presentation [19]. The dosage forms of most herbal preparations possess bioavailability advantage over conventional dosage forms [20]. Also, the dangers of high toxicity and low therapeutic index often shown by certain pure drugs may be eliminated or reduced in a crude formulation. The biflavonoids of *Garcinia kola* are useful in protecting against liver damage. The active fraction “kolaviron” has been patented for commercial exploitation [4]. The physical properties of the tabletted seed and extract have been reported [21]. A cough linctus based on ethanolic extracts from *Garcinia kola* and other plants has been formulated at the International Centre for Ethnopharmacology and Drug Discovery, Nsukka, Nigeria [19]. Syrups are liquid oral preparations in which the vehicle is a concentrated aqueous solution of sucrose or other sugars. As liquid oral solutions, syrups have the advantages that absorption is not delayed while solution takes place in the gut, uniform dosage is certain, and the attractive appearance of a solution in a well-polished bottle has a beneficial psychological effect [22]. Against the background of the wide use of *G. kola* as antimicrobial and for the treatment of several diseases including gastroenteritis, bronchitis, throat infections, colds and cough [6 – 12, 23]; the fact that suitable herbal medicaments could be added to concentrated solutions of sucrose or other sugars to produce syrups [19], and the fact that toxicity is not likely to be a problem [23], this study was undertaken with the objective of evaluating, *in vitro*, the anti-bacterial properties of methanol extract of *Garcinia kola* seed formulated as syrup against *Staphylococcus aureus* and *Escherichia coli* by the standard agar diffusion method.

## MATERIALS AND METHODS

### Reagents

Analytical grades of methanol (Fluka, Germany) and dimethylsulphoxide, DMSO (Merk, Germany) were used for extraction and dilution respectively of the *Garcinia kola* extract. Distilled water (Lion Water, UNN, Nigeria), sucrose and 95% ethanol (Fluka, Germany) were used for preparing the syrup.

### Bacteria media and antibiotic drugs

Nutrient agar (Fluka, Germany) was used as medium for the study. Amoxicillin pure powder (Afrab-Chem. Ltd., Nigeria), metronidazole (Emgyl<sup>®</sup>, Emzor Pharm. Ind. Ltd., Nigeria) and co-trimoxazole (Emtrim<sup>®</sup>, Emzor Pharm. Ind. Ltd., Nigeria) syrups were used as conventional syrup formulations.

### Test microorganisms

Laboratory isolates of *Staphylococcus aureus* and *Escherichia coli* were obtained from stock cultures in the Pharmaceutical Microbiology laboratory, Department of Pharmaceutics, University of Nigeria, Nsukka.

### Collection and identification of plant material

Fresh *Garcinia kola* seeds were obtained in December, 2010 from Nsukka Central market. Authentication of the seeds was done by Mr. A. O. Ozioko of the Bioresources Development and Conservation Programme Center (BDPC), Nsukka, Enugu State, Nigeria and a voucher (PC 98032) specimen is preserved in the Pharmacognosy Herbarium, University of Nigeria, Nsukka.

### Extraction of active principles of *Garcinia kola* seed

The *Garcinia kola* seeds were peeled, cut into pieces, sun dried for two consecutive days and pulverized using an end runner mill. Approximately 350 g of the fine powder was extracted with one liter of methanol by the cold maceration method for 24 h. The extract was further filtered, allowed to evaporate to a semi-solid residue and stored until required for use.

### Preparation of culture media

The growth medium employed was nutrient agar and it was prepared using the method specified in the Oxoid manual.

### Maintenance, activation and standardization of stock microbial cultures

The stock microbial cultures were maintained on nutrient agar slants at 4 °C. In order to activate

these cultures, subcultures were freshly prepared and incubated at 37 °C for 18-24 h before use. Standard suspensions of each test microorganisms were made by transferring a colony from the subculture containing approximately 10 colony forming unit per ml (cfu/ml) of the organisms into 5 ml of sterile distilled water, and adjusting the volume to obtain a cell population of approximately 10<sup>6</sup> cfu/ml [13]. A volume of 0.1 ml of such suspensions was used as inoculum in all the tests.

#### **Preliminary antimicrobial screening**

Preliminary antimicrobial screening of the *Garcinia kola* seed extract was carried out using the cup-plate agar diffusion method [24]. This method depends on the diffusion of antibiotics from holes on the surface of the microbial seeded agar. Molten nutrient agar (20 ml) was inoculated with 0.1 ml of *Staphylococcus aureus* broth culture. It was mixed thoroughly, poured into sterile Petri dishes and rotated for even distribution of the organism. The agar plates were allowed to set and a sterile cork borer (8 mm diameter) was used to bore six holes in the seeded agar medium. Two drops of each of the two-fold dilutions of the extract in DMSO (100, 50, 25, 12.5, 6.25, 3.125 mg/ml) were added into each labeled hole using a sterile pipette. The plates were allowed to stand at room temperature for 15 min to enable the samples to diffuse into the medium before incubating at 37 °C for 24 h. The experiment was repeated for *Escherichia coli*. Three replicate tests were performed in each case. Growth was examined after incubation and the diameter of each inhibition zone was measured and the average determined. A control experiment was also set up against each test organism using DMSO alone without the extract.

#### **Determination of the minimum inhibitory concentration (MIC)**

The MIC of the *G. kola* seed extract was obtained using the agar dilution technique [13]. A stock solution of the extract (100 mg/ml) was prepared by dissolving 1.0 g of the extract in 10 ml of 50 % DMSO (i.e. one part of DMSO in one part of water). Then two-fold serial dilutions were made with sterile distilled water to obtain concentrations between 50 mg/ml and 3.125 mg/ml. A volume of each of the concentrations equal to 0.5 ml was transferred into an agar plate, made up to 20 ml with molten agar and then allowed to set. The surface of the agar was then dried and streaked with isolates. An overnight (24 h) broth culture was used for this experiment. Control plate having 5 ml of 50 %

DMSO in 15 ml of molten agar was prepared for *Garcinia kola*. The plates were then incubated at 37 °C for 24 h. The MIC was taken to be the lowest concentration which showed no visible growth of each of the test isolates on the agar surface. The experiment in each case was carried out in three replicates.

#### **Preparation of syrups and determination of antimicrobial activities**

Syrups were prepared following prescribed standard formulation procedure [25]. *Ab initio*, appropriate quantity of sucrose was dissolved in sterile distilled water and made up to 500 ml. This syrup was used as the vehicle in the formulation. A 1.0 g quantity of *Garcinia kola* extract was weighed and dissolved in 10 ml of 95 % ethanol, placed in the syrup bottle and made up to 100 ml with the vehicle. This method was also used to prepare syrup containing 1 g amoxicillin trihydrate (positive control) and simple syrup which contains no active ingredient (negative control). Two commercial syrups (Emgyl® i.e. metronidazole and Emtrim® i.e. co-trimoxazole) were also used as standards.

The antimicrobial studies on the syrups were carried out using the cup-plate agar diffusion method [26]. Molten nutrient agar (20 ml) was inoculated with 0.1 ml of *Staphylococcus aureus* broth culture. It was mixed thoroughly, poured into sterile Petri dishes and rotated for even distribution of the organism. The agar plates were allowed to set and a sterile cork borer (8 mm diameter) was used to bore five holes in the seeded agar medium. Two drops of each of the syrups were added into the corresponding labeled hole using a sterile pipette. The plates were allowed to stand at room temperature (28 °C) for 15 min to enable the samples to diffuse into the medium before incubating at 37 °C for 24 h. The experiment was repeated for *Escherichia coli*. Three replicate tests were performed in each case. Growth was examined after incubation and the diameter of each inhibition zone was measured and the average determined.

#### **Physicochemical evaluation of the syrups**

##### *Viscosity measurement*

The viscosity of the syrups was evaluated by the Ostwald viscometer (Gallenkamp, Germany) at 25 °C using 20 ml of each sample. Distilled water was used as the standard. Experiments were performed in triplicate for each sample.

##### *Determination of pH*

The pH of each of the syrups was determined using a pH meter (Suntex TS-2, Taiwan) at 25 °C. Three replicate determinations were made for each sample.

## RESULTS

The MIC of the crude methanol extract of *Garcinia kola* against *Staphylococcus aureus* and *Escherichia coli* was evaluated to be  $5.05 \pm 0.12$  mg/ml and  $3.50 \pm 0.04$  mg/ml respectively. The recorded MIC values are the mean of three replicate studies. Table 1 shows the results of the antibacterial effect of different syrup formulations against the test organisms, whereas Table 2 shows the physicochemical properties of different syrup formulations.

## DISCUSSION

The study focused on the antibacterial properties of methanol extract of *Garcinia kola* seed formulated as syrup. The preliminary sensitivity screening shows that the methanol extract of *Garcinia kola* seed possesses activity against *Staphylococcus aureus* (Gram positive bacterium) and *Escherichia coli* (Gram negative bacterium). The result of the preliminary antimicrobial screening is further supported by the MICs of the extract which were  $5.05 \pm 0.12$  mg/ml and  $3.50 \pm 0.04$  mg/ml against *Staphylococcus aureus* and *Escherichia coli* respectively, consistent with earlier reports [6, 10, 11, 13 – 15]. This shows that the active

**Table 1:** Antibacterial effect of the syrup formulations

Sample no	Formulation code	IZD (mm) <sup>a,b</sup>	
		<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
1	GKS	$12.00 \pm 0.05$	$24.00 \pm 0.19$
2	AMX	-	$21.00 \pm 0.07$
3	SIM	-	-
4	COT	$21.00 \pm 0.14$	$27.00 \pm 1.00$
5	MTZ	-	$14.00 \pm 0.13$

<sup>a</sup>Mean  $\pm$  SD, <sup>b</sup>n = 3, IZD = Inhibition zone diameter, GKS= *Garcinia kola* seed syrup, AMX = Amoxicillin syrup, SIM = Simple syrup, COT = Cotrimoxazole syrup, MTZ = Metronidazole syrup, - = No inhibition.

**Table 2:** Physicochemical properties of the syrup formulations

Sample no	Formulation code	Parameter	
		pH <sup>a,b</sup>	Viscosity (cP) <sup>a,b</sup>
1	GKS	$6.73 \pm 0.10$	$5.74 \pm 0.36$
2	AMX	NT	NT
3	SIM	NT	NT
4	COT	$6.80 \pm 0.89$	$8.40 \pm 0.92$
5	MTZ	$6.80 \pm 0.75$	$13.97 \pm 1.50$

<sup>a</sup>Mean  $\pm$  SD, <sup>b</sup>n = 3, cP = Centipoise, GKS= *Garcinia kola* seed syrup, AMX = Amoxicillin syrup, SIM = Simple syrup, COT = Co-trimoxazole syrup, MTZ = Metronidazole syrup. NT = Not tested.

principles of the *Garcinia kola* seed are sensitive to the test organisms. Furthermore, it was noticed that formulating the extract as syrup did not hinder its antibacterial activity, just like the tablet and linctus formulated with *Garcinia kola* in previous studies [19, 21]. The results also indicated that the activity of the *Garcinia kola* syrup compared favourably with that of the extract and demonstrated that the excipients had no negative influence on the drug release properties of the formulation. In other words, the formulation method adopted and the excipients used in the formulation did not have negative effect on the antibacterial activity of the methanol extract of *Garcinia kola* seed; rather it enhanced the presentation of the herbal medicine. Sucrose, a sweetening agent and main content of the simple syrup, was used to mask the bitter taste of *Garcinia kola* seed methanol extract.

As could be seen from Table 1, the syrup containing the extract had an IZD of  $12.00 \pm 0.05$  mm for *Escherichia coli* and  $24.00 \pm 0.19$  mm for *Staphylococcus aureus*, suggesting a good and an effective release of the active constituents from the syrups. On the contrary, the simple syrup prepared according to Pharmacopoeial standard did not inhibit the growth of the test microorganisms, suggesting that the antibacterial effect of the *Garcinia kola* seed methanol extract was not due to the syrup present; rather it was as a result of the active constituents present in the extract. The formulated amoxicillin syrup exhibited strong inhibitory activity against *Staphylococcus aureus* but had no effect against *Escherichia coli* just like metronidazole syrup. The reason for this inactivity is

uncertain but may be related to resistance of the test microorganism to amoxicillin and metronidazole. The development of microbial resistance to commonly used antibiotics has triggered the search for new agents especially from plant sources. Previous studies have shown that GB1, one of the antimicrobial constituents of *Garcinia kola*, had activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and could serve as lead for the development of more effective antibiotics [27, 28]. In addition, co-trimoxazole syrup showed strong inhibitory properties against the test organisms (*Staphylococcus aureus* and *Escherichia coli*), with an IZD that was even greater than the syrup formulation of *Garcinia kola* seed methanol extract. This might be due to synergistic antibacterial effects of sulphamethoxazole and trimethoprim present in co-trimoxazole syrup.

The result of the physicochemical tests performed on the syrup formulations revealed that the pH of the *Garcinia kola* syrup varied with about 0.07 units from the pH of the commercial antibiotic syrups. However, the pH values of the syrup formulations varied insignificantly ( $p > 0.05$ ) from the standard pH value for syrups which is in the range 4.8-5.5 [25]. The pH test result indicated that the syrup formulation of methanol extract of *Garcinia kola* seed is weakly acidic. Table 2 shows that *Garcinia kola* syrup is less viscous than the commercial antibiotic syrups. Compared with the commercial syrups with viscosities of  $8.40 \pm 0.92$  cP for co-trimoxazole syrup and  $13.97 \pm 1.50$  cP for metronidazole syrup, *Garcinia kola* syrup had a viscosity of  $5.74 \pm 0.36$  cP. The observed antimicrobial and physicochemical properties of the herbal syrup could therefore be an added advantage over the commercial syrups in treating infections caused by susceptible micro-organisms.

## CONCLUSION

In this study, a herbal syrup with good physicochemical and antibacterial properties was successfully formulated with methanol extract of *Garcinia kola* seed. Standardization of the *Garcinia kola* syrup will hopefully expose new frontiers by improving on the available safe, stable and efficacious dosage forms of this herbal medicine.

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