



Theoretical and Experimental Investigations on the Antibacterial Activities of Garcinia Kola Seed

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ABSTRACT

The antibacterial activity of *Garcinia kola* seed extract against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumoniae* of was investigated. *Garcinia kola* Seeds were purchased from Okitipupa local market in okitipupa metropolis of Ondo State. The seeds were dehusked, chopped, air dried, pulverized and stored in a clean air tight bottle sample. 100 g of pulverized *Garcinia kola* seed was extracted with 900 mL of analytical grade methanol. The mixture was filtered and concentrated to obtain a crude extract. Agar-gel diffusion inhibition technique was adopted for the antimicrobial screening of *Garcinia kola* seed. 2.0 ml of 50 mg/ml, 75 mg/ml and 100 mg/ml of *Garcinia kola* seed extract was gently poured into each of the wells in the agar plates. Agar plates of ampiclox were also prepared as control. The agar plates of both extract and antibiotic were then incubated at a temperature of 37°C for 24 hours. The zones of inhibition around the well were measured. The zones of inhibition of the extract against selected bacteria range from 00 mm to 21 mm. The control (ampiclox) showed zones of inhibition of 22, 30, 32 and 29 mm against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumoniae* respectively. Based on the zones of inhibition the order of susceptibility of the bacterial to the extract was *Staphylococcus aureus*>*Escherichia coli*>*Bacillus subtilis*>*Klebsiella pneumoniae*. The results obtained from this study, provides scientific evidence that can help in validation of *Garcinia kola* seed as antibacterial agent for the treatment of bacteria infections. Also, the insilico docking study showed that kolaviron have the highest tendency to inhibit all the studied bacterial cell lines.

Keywords: Medicinal plants; *Garcinia kola* seed; Antibacterial activity; Zone of inhibition

INTRODUCTION

Bacterial infections have been a major human problem for decades. Diverse attempt to find antibacterial drug have been made for over 50 years [1]. However, despite the discoveries of many antibiotics, humans are still faced with problems such as multidrug resistance associated with bacterial infections and difficulties in treatment for patients who are allergic to existing antibiotics. [2] The use of medicinal plant extract as a form of treatment of bacterial infections has become popular with the knowledge due to the limitation in the effective life span of antibiotics and over dose which result into microbial resistance [3].

The Nigerian climate supports the growth of many species of plants among which there are various medicinal plants with good antimicrobial potentials [4]. The use of variety of herbal formulations in treatment of ailments such as microbial infections in form of gonorrhoea and skin infections like eczema is rampant among Nigeria traditional herbalist. This has been the practice in Nigeria and many African countries before modern drugs and antibiotics were introduced [5]. Several Nigeria medicinal plants with good antimicrobial properties have been reported [6]. One of such medicinal plants is *Garcinia kola* from the family of the Guttiferae found in most central and western countries of Africa. All the parts of *Garcinia kola* serves as an important constituent of Africa and the world herbal medicine. The reputation of the seed of *Garcinia kola* in Nigeria as poison antidote, anti-hepatotoxic, antioxidant and hypoglycemic cannot be underestimated [7-9]. The usage of antimicrobial agents derived from medicinal plants might provide opportunities to discover novel antibiotics to minimize the challenges of drugs resistance to pathogenic microorganisms [10].

Hence, discovery of novel antibacterial drugs from natural products against infections is highly recommended for development and design of effective, cheap and nontoxic antibacterial agents that could serve as complimentary or replacement to conventional medicines. In view of the vast significance of *Garcinia kola* in herbal medicine. This research is aimed at investigating anti-bacterial properties of *Garcinia kola* seed *via* experimental and theoretical means.

MATERIALS AND METHODS

Sample and Test Organism Collection

Seeds of *Garcinia kola* were purchased from Okitipupa local market in okitipupa metropolis of Ondo State, Nigeria, the seeds were dehusked, chopped and air dried. The cleaned seeds were pulverized and stored in a clean air tight bottle sample. Clinical isolates of *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumoniae* were collected from the Microbiology Laboratory of State specialist hospital Okitipupa, Ondo State, Nigeria. The isolates were properly identified using conventional microbiological techniques [11,12].

Preparation of Extract

100 gms of pulverized *Garcinia kola* seed was transferred into 1L extracting jar. 900 ml of methanol was dispensed into the extracting jar containing the sample. The extracting jar was placed on a mechanical stirrer overnight for proper extraction. The mixture was filtered and the filtrate was transferred into a sterilized beaker. The filtrate was then concentrated on a rotary evaporator at a temperature of 32°C to remove extracting solvent. A dark sticky crude extract obtained was stored in the refrigerator for further investigation.

Antimicrobial Screening of Extract

The antimicrobial screening of *Garcinia kola* seed extract on the selected bacteria was carried out *via* agar-gel diffusion inhibition technique. Sterilized 2.0 mL of a day log phase broth culture of the organism was introduced on the surface of the gelled nutrient agar and was properly spread with a cleaned glass rod [13]. Three (3) wells of 6 mm diameter were aseptically perforated on each agar plate with a clean cork borer, a space of 30 mm distance separating adjacent wells from the plates the edges was ensured. Sterilized insulin syringes was used to seed 2.0 mL of *Garcinia kola* seed solution at concentrations of 50 mg/ml, 75 mg/ml and 100 mg/ml into each of the wells in the agar plates and labeled properly. Similarly, agar plates of ampiclox were also prepared as control. The agar plates of the extract and antibiotic were then incubated at a temperature of 37°C for 24 hours. The diameter of any clear zone of inhibition detected around the agar plates were measured with a transparent ruler as the zones of inhibition. This experiment was repeated for two more times for each extract and the antibiotic used as control.

Insilico Studies Docking Study

The studied molecular compounds (Ligands) were built and optimized with B3LYP at 6-31G* *via* Spartan 14 [14]. The ligands with minimum energy were further subjected to docking study using docking software i.e. Discovery Studio 2017 R2, AutoDockTools-1.5.6, Auto Dock Vina. The optimized ligands were converted to .pdb using discovery studio and the downloaded receptors from <http://www.pdb.org/pdb/home/home.do> were treated by removing ligands and water molecules downloaded with the studied receptors. The treated ligands and receptors were used as input for Auto Dock Tool in order to locate the binding site based on the amino residue in the studied receptors and convert the ligands to .pdbqt. The prepared ligands and the receptors were simulated using Auto Dock Vina and post analysis was carried out using pymol.

RESULTS AND DISCUSSION

The results obtained from the antibacterial screening of *Garcinia kola* seed extract showed in Table 1 revealed that *Garcinia kola* seed extract exhibited strong antibacterial activities against selected bacterial isolates at all concentration 25, 75, 100 mg/ml except in test against *Staphylococcus aureus* at 25 and 75 mg/ml where no zones of inhibition was observed. The zones of inhibition of *Garcinia kola* seed extract against the selected bacteria ranges from 00 mm against *Staphylococcus aureus* at 50 and 75 mg/ml to 21 mm against *Klebsiella pneumoniae* at 100 mg/ml. The control (ampiclox) showed zones of inhibition of 22, 30, 32 and 29 mm against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumoniae* respectively. The zones of inhibition in this study were higher than that reported [9] but lower than that recorded from the study [14,15]. Judging from the zones of inhibition exhibited by the extract, the order of susceptibility of the bacteria to the extract was *Staphylococcus aureus*>*Escherichia coli*>*Bacillus subtilis*>*Klebsiella pneumoniae*.

This suggested that *Garcinia kola* seed extract has the ability to inhibit the growth of any of the selected bacteria at 100 mg/ml. In addition, the extract of the seed of *Garcinia kola* at 100 mg/ml compete favorable with the control (ampiclox). According to the report of (Ezeanya and Daniel 2013) that phytochemical found in plants are responsible for their antibacterial activity. This suggests that the antibacterial activities exhibited by the extract of

Garcinia kola seed against these bacteria might be as a result of the bioactive compounds or phytochemical found in it.

Table 1. Zone of inhibition (mm) of *Garcinia kola* seed extract against the selected bacteria

Clinical isolates bacteria	Zones of Inhibition in (mm) at			Ampiclox (Control)
	50 mg/ml	75 mg/ml	100 mg/ml	
<i>Staphylococcus aureus</i>	0	0	13	22
<i>Bacillus subtilis</i>	8	17	20	30
<i>Escherichia coli</i>	6	14	18	32
<i>Klebsiella pneumoniae</i>	14	19	21	29

Molecular Docking Study

The docking study carried out on series of molecular compounds obtained from *Garcinia kola* seed and sets of bacterial cell line brought about series of binding affinity for individual studied ligand-receptor complexes. This study revealed the molecular compounds in the *Garcinia kola* seed with highest inhibiting tendency. As shown in Table 2, kolaviron have effective composition which enhance its inhibiting ability. Kolaviron possesses the tendency to inhibit all the studied bacterial cell lines (*Bacillus subtilis* (PDB ID: 3zih), *E. coli* (PDB ID: 1ecl), *Klebsiella pneumoniae* (PDB ID: 2rqx) and *Staphylococcus aureus* (PDB ID: 4g8x)) due to highest binding affinity it has in the complex formed with the studied cell lines (Figures 1-4) [16-21].

Table 2. Docking score results for bacterial cell lines

Compound	<i>Bacillus subtilis</i> (kcal/mol)	<i>E. coli</i> (kcal/mol)	<i>Klebsiella pneumoniae</i> (kcal/mol)	<i>Staphylococcus aureus</i> (kcal/mol)
Garcinal	-6	-6.7	-5.3	-5
Garcinoic acid	-6.5	-7.2	-4.6	-5.9
Kolaviron	-6.8	-7.5	-6.3	-7.1
δ -tocotrienol	-5.5	-7.3	-5.3	-5.6

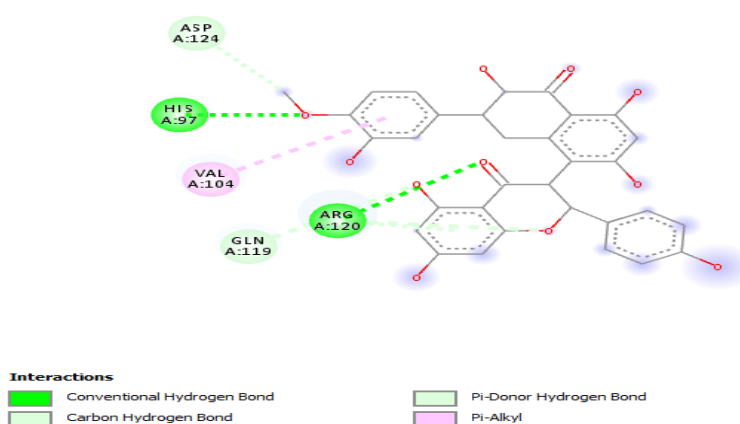


Figure 1. Interactions of Kolaviron with the residue in the active site of *Bacillus subtilis* cell line

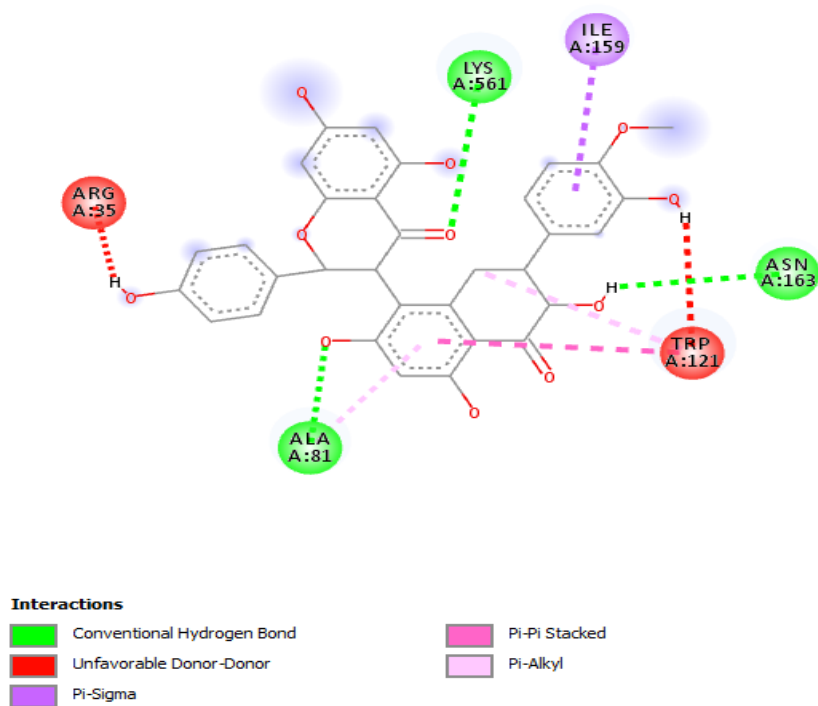


Figure 2. Interactions of Kolaviron with the residue in the active site of *E. coli* cell line

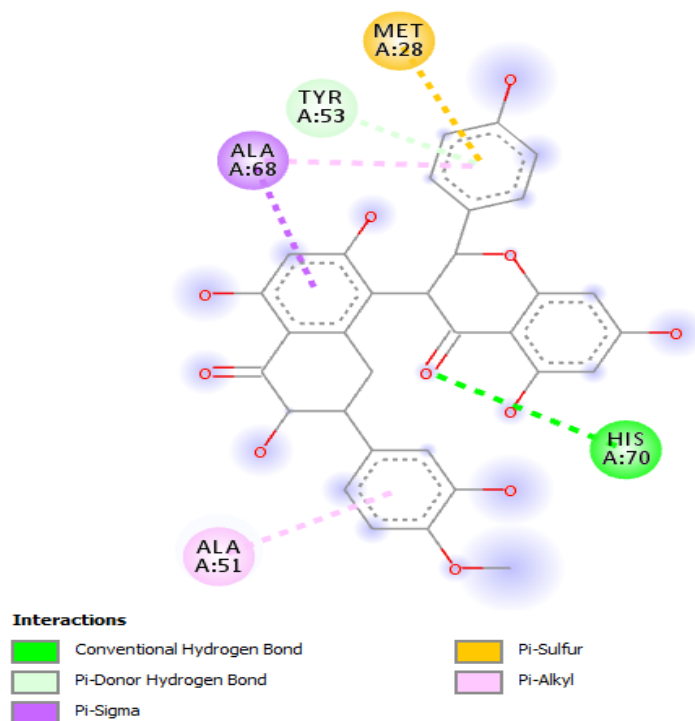


Figure 3. Interactions of Kolaviron with the residue in the active site of *Klebsiella pneumoniae* cell line

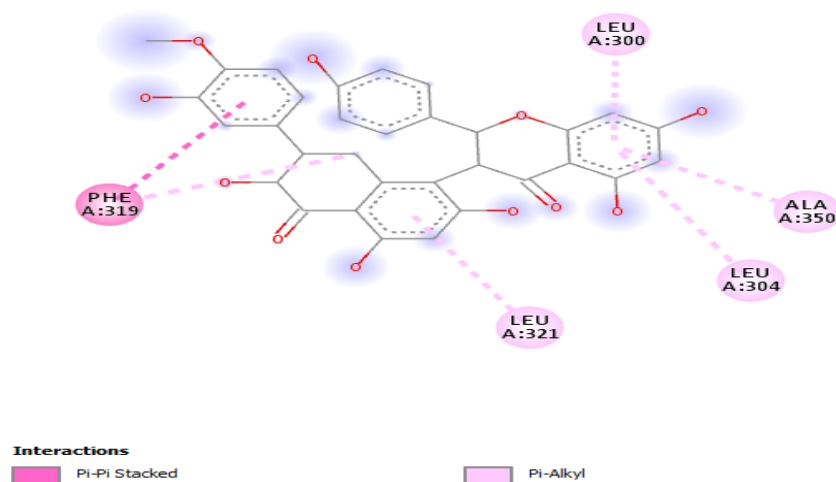


Figure 4. Interactions of Kolaviron with the residue in the active site of *Staphylococcus aureus* cell line

CONCLUSION

This study has ascertained the importance of *Garcinia kola* seed in the treatment of infectious disease caused by bacteria thereby capable of reducing or eliminating problems associated with conventional antibiotics. The antibacterial activities of *Garcinia kola* seed against selected bacterial strains in this study are promising for the development of new antibiotics. Further investigation on the antibacterial activity of isolated compounds from *Garcinia kola* seed is highly recommended for the development and production of novel antibacterial agent with less toxicity for combating infections caused by the test bacteria when used alone or in combination with other antibiotics. Also, the docking study carried out using discovery studio; autodock tool, autodock vina and pymol were effective as a screening tool to identify the compounds in the *Garcinia kola* seed with greatest inhibiting potency. Kolaviron proved to have the highest tendency to inhibit the four studied bacterial cell lines.

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