

Functional Group Analysis and Antibacterial Studies of Column Chromatography Eluates from the Fruit of *Garcinia kola*

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Abstract: The crude extract of *Garcinia kola* fruit was subjected to column chromatographic separation of its component phytochemicals. Three of the eluates obtained gave single spots on a TLC plate. The FTIR characterization of the three eluates revealed that they all possessed alkyl and aromatic hydroxides with dimeric character. Eluates 1, 2 and 3 also possessed amine groups, pyridine/pyrimidine rings and 6-ring ketone moieties respectively.

Antibacterial activity studies of the three eluates showed all to have a cumulative bactericidal activity against five out of the ten organisms used in the study. Eluate 2 which had the pyridine/pyrimidine moiety inhibited the growth of *Klebsiella aerogenes* which was not achieved using the other eluates and the broad spectrum antibiotics Levofloxacin.

Keywords: *Garcinia kola*, eluate, dimeric, pyridine, pyrimidine, *Klebsiella aerogenes*, Levofloxacin.

I. Introduction

Africa and indeed Nigeria is favoured with numerous and readily available plants with medicinal properties. The phytochemical constitution and therapeutic effects of most of these plants are documented. One of these plants is *Garcinia kola* a fruit of a nut-bearing tropical tree native to Nigeria's coastal rainforests [1]. It is also known as bitter kola and consumed all over the country either leisurely or for its medicinal value.

G. kola fruit has been proven to exhibit pharmacological uses in treating coughs, and throat infections [2]. Its stem bark has been shown to contain a complex mixture of phenolic compounds such as tannins, guttiferin [3], biflavonoids, xanthenes, benzophenone, kolaflavanone and garcinia flavanone [4] all of which have antimicrobial activity. In addition, the plant possesses hepatoprotective [5,6], analgesic and hypo-glycemic activities [7,8]. In traditional medicine, it has a reputation in the management of sickle cell disease and as poison antidote [9,10].

In 1999, a team of researchers at the Division of Experimental Therapeutics of Walter Reed Army Institute of Research, Washington D.C., screened about 2, 000 compounds against Ebola and other viruses which seem to have no treatment. They discovered that a compound from *G. kola* was able to arrest the replication of the Ebola virus [11]. A dimeric flavonoid isolated from the fruit was found to be responsible for this observed activity [12].

The antimicrobial activity of the fruit extract of *G. kola* has been revisited in this study. Column chromatography eluates were tested for activity against a variety of bacteria strains and functional groups from potent eluates were identified.

II. Materials and Method

1.2.1 Chemical reagents

Absolute ethanol, chloroform, Petroleum ether and methanol were of high purity and obtained from Finlab Owerri, Nigeria. All other chemicals were of analytical grade and obtained from standard chemical suppliers.

1.2.2 Plant material

Fresh fruits of *G. kola* were bought from Ekeonunwa market in Owerri, Nigeria and identified at the Plant Science and Biotechnology Department of Imo State University Owerri. The fruits were peeled to remove the shell covering the pulp which was then chopped to small pieces and air-dried. Thereafter, the dried pulp was blended using a manual blender and the powdered sample was stored in a polythene bag and placed at room temperature until they were used.

1.2.3 Preparation of fruit extracts

Hundred grams (100 g) of powdered fruit sample were weighed in a 500 ml beaker and 300 ml of absolute ethanol (98 %) was added to the beaker containing the powdered sample and left to stand for 72 h at room temperature. Thereafter, the mixture was filtered with a white cotton cloth, and then refiltered using a Whatman filter paper (No. 1). The filtrate was concentrated to a gel form at 35 °C using a water bath. The concentrated extract now dark brown in colour, was partitioned in 1:1 chloroform-water mixture in a separatory funnel. The lower chloroform layer was collected and allowed to dry at room temperature. It was stored in a refrigerator until required.

1.2.4 Column Chromatographic Separation

The column was packed with silica gel 60 – 200 mesh using the slurry method. 1 g of the chloroform extract was redissolved in 20 ml of chloroform and 10 g of silica gel was added and mixed thoroughly with the plant matter. The system was then allowed to dry to a free flowing powder. It was then introduced into the loaded column and eluted with 200 ml each of the following solvent mixtures: Petroleum ether-Chloroform (1:0, 9:1, 4:1, 7:3, 3:2, 1:1, 2:3, 3:7, 1:4, 1:9 0:1 v/v) and Chloroform-Methanol (9:1, 4:1, 7:3 v/v). The eluting solvent was collected at 100 ml volumes and each allowed to evaporated to about 5 ml volume at room temperature to concentrate any eluted compound. Each concentrate was then spotted on TLC plates. After developing the plates, eluates that gave a single spot were characterized and screened for antimicrobial properties.

1.2.5 Characterization of Eluates

Eluates were analysed at National Research Institute for Chemical Technology (NARICT) Zaria Nigeria, using FTIR- 8400S Fourier Transform Infrared Spectrophotometer by SHIMADZU.

1.2.6 Test Organisms

The bacteria strains used for this study were collected from the microbiology Department of Federal Medical Center Owerri. The bacteria strains were cultured and maintained as described by Cruickshank et al [13]. The isolates were identified using biochemical methods as described by Holt et al [14]. The bacteria strains were identified to be *Bacillus* spp, *Clostridium perfringens*, *Escherichia coli*, *Proteus mirabilis*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus agalactiae*, *Enterococcus faecalis* and *Klebsiella aerogenes*.

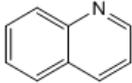
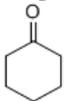
1.2.7 Antimicrobial Activity Studies

The elutes with single spots on TLC plates were allowed to dry at room temperature. Each was then redissolved using five drops of dimethyl sulphoxide (DMSO) and concentrated to 10 mg/ml before been applied on sterile filter paper discs which were allowed to dry. 0.2 ml of a 24 h broth culture of each of the bacteria species were spread on the surface of gelled sterile Muller-Hinton plates. The paper discs adsorbing different eluates were placed at different areas on the surface of each plate. The plates were incubated at 37 °C for 24 h. The diameter of the growth free zones around the respective discs were measured and recorded. Levofloxacin which inhibited the growth of most of the studied organisms was used as control.

III. Results and Discussion

Column chromatography eluates after spotting on TLC plates and developed, gave three isolates with single spots on the plates. Their order of elution, eluting solvent mixtures, colour and functional groups of these isolates are summarized in table 1.

Table 1: Characterization table of column isolates from Garcinia kola fruit

Order of Elution	Solvent Mixture	Colour of Eluate	Absorbance Range (cm ⁻¹)	Relative Intensity	Functional Group
1	Petroleum ether 1:0	Cream	3595-3425	w,br	R-OH, Ar-OH, Dimeric (having intramolecular hydrogen bonding)
			1660-1610	w	Amino acid containing NH ₂ group
2	Petroleum ether-chloroform 4:1	Yellow	3595-3425	s,br	R-OH, Ar-OH, Dimeric (having intramolecular hydrogen bonding)
			1650-1580	m	Pyrimidines,  Quinolines 
3	Chloroform 1:0	Orange	3595-3425	m,br	R-OH, Ar-OH, Dimeric (having intramolecular hydrogen bonding)
			1695-1660	s	6-ring ketone 

Key: br = broad, m = medium, s = strong, w = weak

Alkyl and aromatic compounds with hydroxyl groups abound in natural products. The absorbance of 3595-3425 cm⁻¹ in the spectra of the three eluates showed their presence in all the fractions and exhibiting a dimeric character. The existence of a dimeric flavonoid has earlier been shown to be found in the fruit of *G. kola* [12].

Eluate 1 gave a weak absorption peak at the range of 1660-1610 cm⁻¹ for an amine derivative. Eluate 2 gave a medium absorption peak within the range of 1650-1580 cm⁻¹ indicating the presence of pyridines and/or quinolines existing independently or as moieties of larger compounds. The presence of 6-ring ketones in eluate 3 was shown by a strong absorption peak at the range of 1695-1660 cm⁻¹. This ketone ring is likely to be a part of a flavonoid compound.

The results obtained from the antimicrobial activity tests of the isolates are summarized in table 2.

Table 2: Antimicrobial activity test results of isolates from Garcinia kola fruit

Microbes	Diameter of Zone of Inhibition (mm)			
	Eluate 1 (10 mg)	Eluate 2 (10 mg)	Eluate 3 (10 mg)	Levofloxacin (20 mcg)
Bacillus spp	–	–	–	8
C. perfringens	–	–	–	6
E. coli	–	–	–	6
P. mirabilis	4	4	–	4
S. pyogenes	2	–	–	6
S. aureus	–	6	–	6
P. aeruginosa	–	–	–	4
S. agalactiae	–	–	4	6
E. faecalis	–	–	–	16
K. aerogenes	–	6	–	–

Eluate 3 was active only on the organism *S. agalactiae* which was resistance to the other two eluates used in the antibacterial study. This organism was however more sensitive to the control drug Levofloxacin even though eluate 3 was at higher concentration. The antibacterial activity of eluate 3 might be viewed from the 6-ring ketone found in its scan. The lactones are the likely relatives of this observed family and are found in

bioactive compounds like ascorbic acid and antibiotics like erythromycin. Worthy of note is that the control drug Levofloxacin also has a 6-ring ketone as part of the drug molecule.

The activities of eluates 1 and 2 were either similar to or lower than that shown by the control drug. Eluate 2 however showed significant activity against *K. aerogenes* which was resistant to the control drug. *Klebsiella* organisms are responsible for a wide range of disease states notably pneumonia, diarrhea, urinary tract infections, meningitis and soft tissue infections [15]. The scan of eluate 2 identified the presence of pyridines and/or quinolines which are alkaloids and form the nuclei of over 7, 000 existing drugs [16]. Their presence in this eluate possibly as a nucleus of a larger molecule could have been responsible for its activity against *K. aerogenes*.

IV. Conclusion

Column chromatography eluates of the fruit of *G. kola* were shown to possess antibacterial activity reasserting the therapeutic effect of this fruit. FTIR scans of the eluates showed that the active components possess alkyl and aryl hydroxides which have dimeric properties, amines and compounds with pyridines and/or quinolines nuclei. A compound in the Petroleum ether- Chloroform eluate inhibited the multiplication of *K. aerogenes* which was not achieved using Levofloxacin. This compound is likely to be of a pyridine and/or quinoline origin.

Sponsorship

Self sponsored. All authors have approved this final article.

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