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COMPARATIVE IN VIVO INHIBITORY EFFECT OF *GARCINIA KOLA* SEED PARTITIONED FRACTIONS ON THE GASTROINTESTINAL MICROFLORA IN MALE ALBINO RATS

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ABSTRACT

Introduction: *Garcinia kola* (Heckel), from the family Gluciaceae, is one of the numerous medicinal plants of high significance in traditional medicine practice and nearly all parts of the tree have economic importance. The seeds are used in the treatment of cough, diarrhoea, menstrual cramp, gonorrhoea, bronchitis, gastroenteritis, hepatitis and is also used as aphrodisiac. **Aim**: The Comparative *in vivo* inhibitory effect of *Garcinia kola* seed partitioned fractions on the gastrointestinal microflora in male albino rats, was studied using the aqueous and chloroform fractions from methanol crude extract with the goal of establishing their antimicrobial effects comparatively using viable plate counts. **Methods**: The seeds of *Garcinia kola* were purchased, peeled, washed, shredded into tiny pieces, sun-dried, pulverized, defatted using petroleum ether, the marc was air-dried, followed by cold maceration with methanol for 72 hours, filtered and evaporated to dryness to obtained the extract. The methanol crude extract was successively partitioned to obtain the aqueous and chloroform fractions. They were evaporated to dryness and stored in sample bottles in a refrigerator at 4^{o}C. After acclimatization of the experimental rats, an in vivo microbiological

assay was carried out on eighteen male albino rats, divided into six groups of three rats per group, three groups for aqueous fraction and chloroform fraction respectively, the seventh group was used as untreated controls and they were separately kept, food and water were made available to them *ad libitum*. The baseline (initial) levels of microorganisms in their faeces (500mg) were determined using viable plate counts method of the bacterial colonies (cfu/mg). The plates were incubated at 37°C for 16-18 hours and the colonies that took up stains (pink) were counted and recorded in the colonies forming unit per milligram (cfu/mg). These animals were then orally administered with the extract fractions of 350mg/kg/day, 1,500mg/kg/day and 2,000mg/kg/day doses according to their groups for 7 days. The faecal sample of each rat was taken every two days for antimicrobial analysis as in day zero to determine the level of reduction of the bacterial load of the Gastrointestinal tract (GIT) of the rats. **Results**: The results showed that aqueous fraction had more significant reduction in the colony-forming unit per milligram of the faeces in the bacterial colony counts than the chloroform fraction. The reductions were dose dependence. Differences between means were compared using students t-test statistical analysis. These differences between means were significant at 95% confidence limit ($P<0.05$). **Conclusion**: From the statistical analysis the reductions in the bacterial load of the GIT of the rats were due to the administered extract fractions.

KEYWORDS: Acclimatization, *Ad libitum*, Antimicrobial, Colony, *Garcinia kola*, Inhibitory, Microflora, Microorganism.

INTRODUCTION

Throughout the ages, humans have relied on nature for their basic needs, for the production of food, shelter, clothing, transportation, fertilizers, flavours, fragrances and medicines.[1] Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies. The use of medicinal plant has attained a commanding role in health care system all over the world. This involves the use of medicinal plant not only for the treatment of diseases but also as a potential material for maintaining good health and conditions.[2]

Garcinia kola is one of the numerous medicinal plants of high significance in traditional medicine practice. It belongs to the family Cluciaceae. The family contains about forty genera and one thousand species, which include trees, shrubs or lianas. The main genera are *Hypericum*, *Kielmeifera*, *Clusia*, *Calophylum* and *Garcinia*. The genus *Garcinia* contains

about four hundred species [3]. *Garcinia kola* (Heckel) tree is about 6 meters to 9 meters high with big shiny leaves 8-15cm long. It is a tree that grows wild and is sometimes planted in villages, most commonly in moist environments. The plant is wide spread throughout West Africa and is found up to an elevation of 900 meters.[4] When the seeds are chewed, they possess a bitter astringent and resinous taste, followed by a slight sweetness. Unlike the seeds of the common kola (*Cola nitida*) which has both central nervous system (CNS) stimulant and blood pressure elevation effects [5]. 'Bitter kola' has no central nervous system effect.[4]

Ethnomedicinally, the seeds are used to prevent or relieve abdominal colic, treatment of cough, asthma, bronchitis, gastroenteritis, rheumatism, menstrual cramp, hepatitis and diarrhoea.[6,7] The roots are used for treatment of tooth decay and gonorrhoea.[8]

Moreover, *Garcina kola* seed extract has been shown to cause histological and biochemical alterations such as testicular atrophy.[9] and alterations of the gonadal hormones and pituitary gonadotrophin levels in serum.[10] It contains alkaloid, flavonoid and resin.[11,12,4,13] The seeds also contain Nitrogen, phosphorus, potassium, magnesium, calcium, sodium and sulphur.[14] The kolaviron, flavonoids, bioflavonoids and kolaviron have antidiabetic, anti-hepatotoxic, antimicrobial and anti-inflammatory effects respectively [15,16,12,11] . The seeds are also used as aphrodisiac and poison antidote[17]

Figure 1: Showing a collection of *Garcinia kola* seeds.

Phylogeny of *Garcinia kola* (Scientific Classification) According to Angiosperm Phylogeny Group (APG) System.[18]

Kingdom: Plantae

Clade: Angiosperms

Clade: Tracheophytes

Clade: Eudicots

Clade: Rosids

Order: Malpigiales

Family: Cluciaceae

Genus: *Garcinia*

Species: *G. kola* Heckel

Common Name: Bitter kola

Local Names: Ibibio: Efiat, Efik: Efiari, Igbo: Adi, (Awka)-Ugugolu, (Owerri)-Aku ilo, Yoruba: Orogbo, Hausa: Gida goro

MATERIALS AND METHODS

Collection of *Garcinia Kola* seeds

The fresh seeds of *Garcinia kola* were purchased at Itak Ikot Akab Village in Ikono Local Government Area of Akwa Ibom State, Nigeria.

Extraction of *Garcinia kola* Seeds

The seeds were peeled to remove the testa, washed and shredded into tiny pieces, sun-dried and pulverized. The powder was defatted with petroleum ether, the marc was air-dried and macerated in methanol for 72 hours, filtered and concentrated to thick extract. The methanol crude extract was partitioned with distilled water and chloroform. The solvents partitioned fractions were concentrated to dryness, weighed and stored in a refrigerator at 4°C.

Baseline experimental tests on the faeces of the laboratory animals on day zero

After acclimatization, rats were kept separately in seven groups (3 rats in each group) food and water were made available to them *ad libitum*. A baseline examination of their faecal cultures were carried out using viable plate counts method of the bacterial colonies (cfu/mg) for all the groups to determine the baseline or initial level of microflora load of the gastrointestinal tract (GIT) before administration of the partitioned fractions to the experimental animals in six groups, the seventh group served as the untreated controls.

Preparation of McConkey agar media

McConkey agar of 5.8mg was dissolved in 100mL of distilled water and sterilized in autoclave for 15 minutes, then allowed to cool, at 45°C the molten agar was dispensed into the bacteria inoculated petri dishes.[19]

Viable plate counts: an aliquot of the faecal sample (500mg) of each rat was suspended in 50ml of sterile normal saline to make a 10-fold dilution and a concentration of 10mg/mL. An

aliquot of 0.1mL of the resulting suspension was inoculated into each sterile 15cm petri dish and 25mL of molten McConkey agar at 45°C was poured in, then swirled gently to mix properly. The plates were incubated at 37°C for 16-18 hours and the colonies that took up stains (pink) were counted and recorded in colonies forming unit per milligram (cfu/mg). 0.1mL of 10mg/mL suspension is equivalent to 1mg/mL.[20]

Preparation and Administration of Solvent Partition Fractions to the Laboratory Animals

Each of 350mg/kg/day, 1500mg/kg/day and 2000mg/kg/day[9] of the aqueous(GKA) and chloroform (GKC) solvent partition fractions were dissolved in 1mL to 2mL of distilled water using 20% tween 80 as a solubilizing agent. After the baseline (initial) faecal examination on the bacterial colonies count of each rat on day zero, the drugs were orally administered to the rats for seven days.

In vivo antimicrobial evaluation of the aqueous (GKA) and chloroform (GKC) fractions on 18 male albino rats

After the daily oral administration of each solvent partition fraction (the aqueous and chloroform fractions) to the animals according to their groups and doses, faecal examination was carried out every two days using viable plate count as in the baseline experimental test from day 2 to day 8. The resulting bacterial colonies were counted and recorded in colonies forming unit per milligram (cfu/mg).

Statistical Analysis

Data were presented as mean±SEM. Differences between means were compared using students t-test statistical analysis. These differences between means were accepted at 95% confidence limit ($P<0.05$).

RESULTS

The in vivo inhibitory effect of *Garcinia kola* seed aqueous (GKA) and chloroform(GKC) partitioned fractions on the gastrointestinal microflora in male albino rats were presented in tables 1 and 2. Figures 2 and 3 show graphical representation of the means (±standard Error mean SEM) of bacterial colony counts of the faecal matter of animal groups treated with the extract fractions for 7 days. The reductions in the bacterial colony count for the administered oral doses were significant at ($P< 0.05$) when compared with untreated controls (UNTC). The deductions were dose dependence.

Table 1:

Bacterial colony count/ mg faeces (cfu/mg)					
	DAY 0	**DAY 2**	**DAY 4**	**DAY 6**	**DAY 8**
UNTREATED CONTROL	111 ± 0.16(3)	108 ± 0.44(3)	110 ± 0.11(3)	105 ± 0.22(3)	109 ± 0.18(3)
GK-A TREATED 350mg/kg/day	81 ± 0.71(3)	75** ± 0.49(3)	68** ± 0.36(3)	57** ± 0.13(3)	46** ± 0.21(3)
1500mg/kg/day	98 ± 0.60(3)	86** ± 0.29(3)	62** ± 0.31(3)	24** ± 0.16(3)	6** ± 0.19(3)
2000mg/kg/day	101 ± 0.37(3)	56** ± 0.25(3)	21** ± 0.20(3)	11** ± 0.15(3)	2** ± 0.10(3)

Data presented as means ± standard errors of mean. Number in parenthesis represents number of animals per treatment group. ** Significant ($P < 0.05$) difference of microflora reduction from untreated controls.

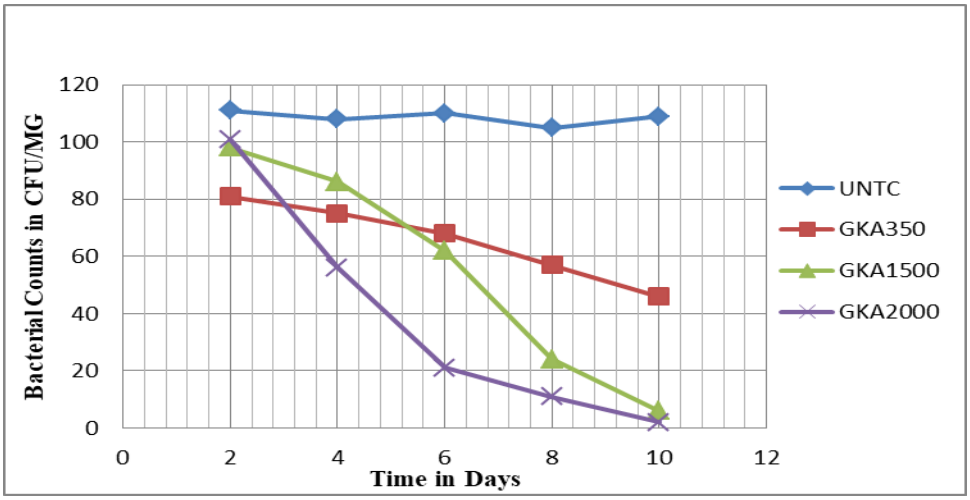

Figure 2: Graphical representation of bacterial colony counts of faecal samples of animals treated with *Garcinia kola* seed aqueous (GKA) fraction

Table 2:

Bacterial colony count/ mg faeces (cfu/mg)					
	DAY 0	**DAY 2**	**DAY 4**	**DAY 6**	**DAY 8**
UNTREATED CONTROL (UNTC)	111 ± 0.16(3)	108 ± 0.44(3)	110 ± 0.11(3)	105 ± 0.22(3)	109 ± 0.18(3)
GK-C TREATED 350mg/kg/day	106 ± 0.50(3)	98** ± 0.31(3)	92** ± 0.66(3)	86** ± 0.43(3)	67** ± 0.21(3)
1500mg/kg/day	76 ± 0.34(3)	69** ± 0.42(3)	51** ± 0.55(3)	35** ± 0.31(3)	22** ± 0.18(3)
2000mg/kg/day	97 ± 0.41(3)	71** ± 0.34(3)	54** ± 0.51(3)	32** ± 0.28(3)	19** ± 0.16(3)

Data presented as means ± standard errors of mean. Number in parenthesis represents number of animals per treatment group. ** Significant ($P < 0.05$) difference of microflora reduction from untreated controls.

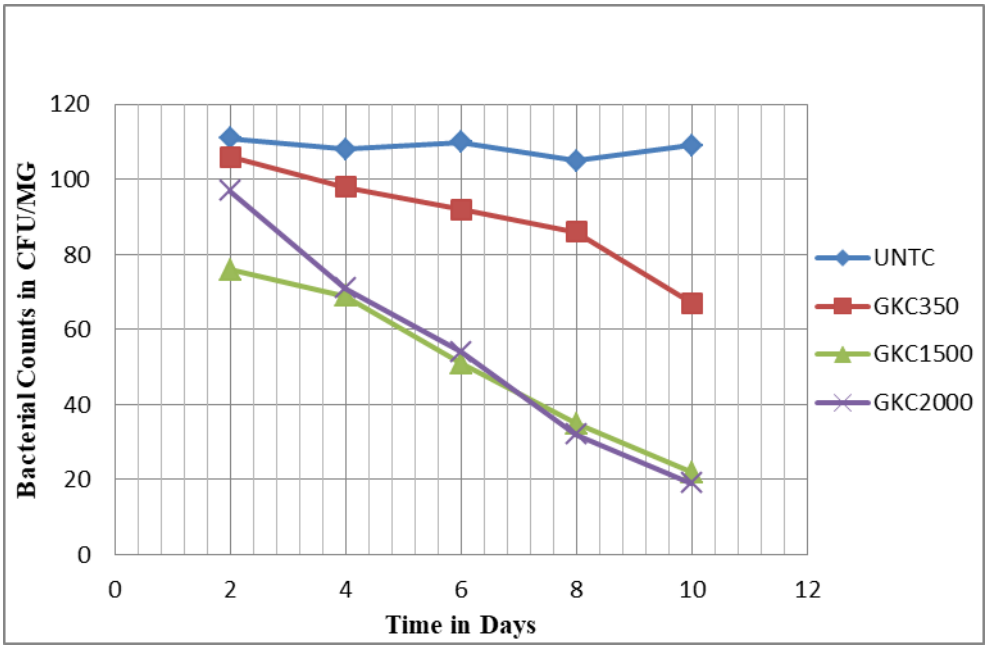

Figure 3: Graphical representation of bacterial colony counts of faecal samples of animals treated with *Garcinia kola* seed chloroform (GKC) fraction.

DISCUSSION

Plants even though are served as source of food, drug and shelter, but also serve as active larvicides/ insecticides.[21] The use of medicinal plants has attained a commanding role in health care delivery system all over the world. This involves the use of medicinal plants not only for the treatment of diseases but also as potential material for maintaining good health and conditions[2] Testing of microorganisms for antimicrobial is a common laboratory procedure that serves as an important tool to chemotherapeutic intervention during cases of infections. On the inhibitory effect of *Garcinia kola* seed partitioned fractions (GKA and GKC) on the gastrointestinal microflora in male albino rats, the in vivo antimicrobial evaluation was done using Viable Plate Counts Method.[20] The results of this research work indicated that these fractions of *Garcinia kola* seeds exhibited potent in vivo antimicrobial effects and this inhibitory activity of the drugs were due to the phytochemical components which were highly effective against microflora in the GIT. The comparative in vivo antimicrobial research carried out on these fractions of the methanol crude extract of *Garcinia kola* afforded the observation that there was a significant bioactivity of the aqueous

fraction compared to that of chloroform fraction measurable in the significant reductions of bacterial colony counts of the faecal matter for the administered oral doses on the male albino rats, when compared to the untreated controls as shown in figures 2 and 3 by graphical representations of the mean (± standard error of mean) of the bacterial colony counts of the faecal matter of animal groups treated with the partitioned fractions for 7 days. Moreover, these results have proven the ethnomedicinal use of *Garcinia kola* seeds for the treatment of various diseases of bacterial origin.

CONCLUSION

This research work was done to compare antimicrobial efficacy or potency of the aqueous and chloroform fractions of the methanol crude extract from the seeds of *Garcinia kola*, designated GKA and GKC respectively. This work has revealed that both fractions had significant potent antimicrobial activity with aqueous fraction being more active than the chloroform fraction. This showed that the aqueous fraction had more inhibitory effects on the gastrointestinal microflora of the male albino rats. These results established the pharmacological basis of the therapeutic application of *Garcinia kola* seeds in traditional medicine for the treatment of cough, diarrhoea, gonorrhoea and gastroenteritis. Furthermore, it is hereby recommended that the chromatographic isolation of the active component(s) in the aqueous (GKA) and chloroform (GKC) fractions that caused significant reductions in the bacterial colony counts for the administered oral doses should be carried out.

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