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In-Vitro Antibacterial Activity of Crude Ethanol and Aqueous Garcinia Kola Seed Extracts on Selected Bacterial Isolates

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Abstract **– The study was conducted to screen in-vitro antibacterial activity of crude ethanol and aqueous extracts of Garcinia kola seed against some selected bacterial isolates composing of Gram positive and Gram negative organisms namely *Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa.* The disc diffusion method was employed to determine the inhibitory effects of the seed extract on the test micro-organisms. The ethanol and aqueous extract of the seed at concentration of 4g/40ml were diluted at the concentration of 0.1g/ml, O.2g/ml, 0.4g/ml, 0.6g/ml and 0.8g/ml, then impregnated into a sterile filter paper disc. Also ethanol alone was impregnated into the sterile filter paper disc and used as control. The zones of inhibition obtained for ethanol extract (4g/40ml) of Garcinia kola seed ranged between 12mm-15mm. The zone of inhibition obtained for diluted ethanol extract (4g/40ml) of Garcinia kola seed at concentration 0.1g/ml, 0.2g/ml, 0.4g/ml, 0.6g/ml and 0.8glml ranged from 8mm-15mm also the zone of inhibition obtained for ethanol extract (40g/80ml) of Garcinia kola seed ranged between 12mm-16mm, the zone of inhibition obtained for diluted ethanol extract (40g/80ml) of Garcinia kola seed at concentration 0.1g/ml, 0.2g/ml, 0.4g/ml, 0.6g/ml and 0.8g/ml ranged from 7mm-15mm. the aqueous extract (4g/40ml and 40g/80ml) of Garcinia kola seed and the diluted aqueous extract were all resistant to *Staphylococcus aureus, Escherichia Coli and Pseudomonas aeruginosa*. The zone of inhibition of *Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa* at concentration of 4g/40ml, 40g/80ml, 0.1g/ml, 0.2g/ml, 0.4g/ml and 0.8g/ml ranged from 9mm-16mm, 8mm-13mm, 17mm-12mm respectively, the zone of inhibition of ethanol alone ranged between 8mm-10mm. This investigations shows that Garcinia kola is effective only when used in organic solvent at higher concentration.**

Keywords **– Garcinia Kola, Antibacterial, Staphylococcus Aureus, Escherichia Coli and Pseudomonas Aeruginosa.**

I. INTRODUCTION

Historically, plants have provided a source of inspiration for novel drug compounds, large contribution to human health and well-being. Their role is two ways in the development of new drug; They may be become the basis for the development of new medicine i.e. a natural blue print for the development of new drugs or a phyto medicine to be used for the treatment of disease though there is availability of various orthodox drugs for the treatment of respiratory track diseases in Nigeria, there is increase in the search for herbal remedies [1].

The seeds of Garcinia Kola forms a major part of the herbal preparation used in traditional African medicine practice for the treatment of various respiratory tract disease.

Garcinia kola Heckel (Gutti ferare, Bitter kola) is one of the useful indigenous trees in Nigeria and in West and central Africa [2]. It is known as orogbo in Yoruba land, Namijin-goro among the Hausas, Akuilu in Igbo land. Zhila-goro in Zalicva. It is popular in Southern Nigeria, the plant is extensively used in herbal medicine and as food. It is usually found in the tropical rain forest region of West Africa, it prevails as a multi-purpose tree crop in the home gardens of Southern Nigeria [3]. The tree is usually cultivated within villages in Southern Nigeria. It grows to a height of about 12-14m and produces reddish, yellowish or orange coloured fruit [4]. Each fruit contain 2 to 4 yellow seeds and a sour tasting pulp, the seed when chewed have a bitter astringent taste, the flowering of the plant occur between December and January while the fruit mature between June and August.

Garcinia kola is highly valued because of its medicinal uses [5]. The seed are chewed as an aphrodisiac or used to cure cough, dysentery, chest colds in herbal medicine. Garcinia kola could serve as a raw material for pharmaceutical industries [6]. Garcinia kola is used in folklore remedies for the treatment of ailment such as liver disorder, hepatitis, diarrhoea, laryngitis, bronchitis and gonorrhoea [7]. The seed is used to prevent and relieve colic, can as well be used to treat headache, the plant has also found usefulness in the treatment of stomach ache and gastritis. Garcinia kola has the capacity to eliminate harmful bacteria such as Escherichia Coli, Staphylococcus aureus and Pseudomonas aeruginosa because of its anti bacteria properties. Garcinia kola has been shown to inhabit smooth muscle activity, it relaxes the smooth muscles of the uterus and the intestine its alkaloid and bioflavonoid fraction are said to relax the smooth muscles. It is believe in my community that Garcinia kola drives evil spirit and snakes away, also in the treatment of cough and entertainment of visitors during ceremonies.

The aims and objectives of this work are to investigate the antimicrobial effect of garcinia seed extract on selected bacterial isolates, to compare the effect of crude ethanol extract and Aqueous extract of garcinia kola seed on selected bacterial isolates and to investigate the effect of the different dilution and concentration extracts on selected isolates.

II. MATERIALS AND METHODS

Study Area

The plant sample was purchased from mile 1 Market Port Harcourt, Rivers State Nigeria, they were purchased fresh and packed in clean polythene bags and transported to the botanist for identification. The seed were identified

by a botanist in the department of applied and Environmental Biology, faculty of Science, Rivers State University of Science and Technology Port Harcourt Rivers State Nigeria. The research work was carried out in the diagnostic Laboratory of Medical Laboratory Science Department in the Faculty of Science, Rivers State University of Science and Technology, Port Harcourt.

Preparation of Seed Extracts

The seeds were dried under room temperature for 7days and then grounded into fine power using sterilized pestle and mortar.

Crude Aqueous Extraction

2g of the fine powder of Garcinia kola was weighed and soaked in 20ml of distilled water and left overnight. Another 40g was weighed and soaked in 80ml of distilled water also left over night. The infusion was filtered off with sterile filter paper (Whatman No.1). The extract were then stored at 4^0c for further analysis.

Crude Ethanolic Extraction

4g of the fine powder of Garcinia kola was weighed and soaked in 40ml of 95% ethanol left overnight at room temperature. Another 40g was weighed and soaked in 80ml of 95% ethanol and left overnight at room temperature. It was filtered using sterile filter paper (Whatman No.1) then transferred into reagent container and was preserved in the refrigerator for further usage.

Test Organism Used

The pure culture of bacterial isolates of pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli were obtained from the Braithwaite Memorial re-identified and subcultured on nutrient agar and incubated overnight at 37^0c and preserved in the refrigerator at 4^0c until required for analysis.

Inoculation of Test Organisms

With a bursen flame, the wire loop used was sterilized in the flame before it was used to pick a colony of staphylococcus aureus form already subculture plates; it was inoculated onto a plate of nutrient agar and streaked evenly over the entire plate. The loop was flamed again to sterilize, then used to pick another colony of staphylococcus aureus and inoculate on to a second plate of the nutrient agar, and was streaked evenly over the entire plate. The loop was flamed again to sterilize and used to pick a colony of pseudomonas and inoculated onto a plate of nutrient agar and streaked evenly over the entire plate, the loop was flame to sterilize and another colony of pseudomonas aeruginosa was picked and inoculated over the entire plate. The wire loop was flamed again to sterility and used to pick a colony of Escherichia coli, inoculated over the entire agar plate, the wire loop was flamed again, then used to pick another colony of Escherichia coli and inoculate over the entire plate.

Antimicrobial Screening Test

The disc diffusion method was used to determine the inhibitory effects of the seed extract against clinical isolates. A filter disc of 6mm in diameter was made using a perforator then put in boujou bottle and sterilize in the autoclave for 121^0c at 15minutes, from the first concentration of 4g/40ml serial dilution was prepared for both aqueous and ethanolic extracts in the order of the following concentration. 0.1g/ml, 0.2g/ml, 0.4g/ml, 0.6g/ml and 0.8g/ml respectively.

From the second concentration of 40g/80ml the same serial dilution was prepared as above. 0.1ml (10ul) was impregnated into the filter disc of 6mm in diameter each for 72 filter paper with the use of sterile forceps. The filter paper containing vary concentration of both aqueous and ethanolic extract were placed on the surface of each plate seeded with the test organisms, another filter paper containing only ethanol was also place on the plate seed with the test organism used as control. The plates were incubated at 37^0c for 24 hours. Antimicrobial activities of the extracts were determined by measuring clear zone of inhibition with ruler.

III. RESULT

The antibacterial effects of Garcinia kola seed extract on the test organisms used at varying concentrations are shown in the figure below.

Fig.1: The in-vitro antibacterial sensitivity of ethanol extract (4g/40ml) of Garcinia kola seed on selected clinical isolates. After ethanol alone was used as control showed zone of inhibition as follows , Staphylococcus aureus 10mm, Escherichia coli 10mm and pseudomonas aemginosa 8mm ethanol extract zone of inhibition, staphylococcus showed aureus 15mm, Escherichia coli 13mm, pseudomonas aeruginosa 12mm. it also different zones of inhibition at varying diluted concentration of 0.1g/ml, 0.2g/ml, 0.4g/ml, 0.6g/ml, and 0.8g/ml, showed 8mm, 9mm, 10mm, 10mm, 12mm, 14mm for staphylococcus aureus. Escherichia coli had 8mm, 9mm, 10mm, 11mm, and 12mm. lastly psendomonas aeruginosa had 8mm, 8mm, 9mm, 10mm, 11mm and 12mm respectively.

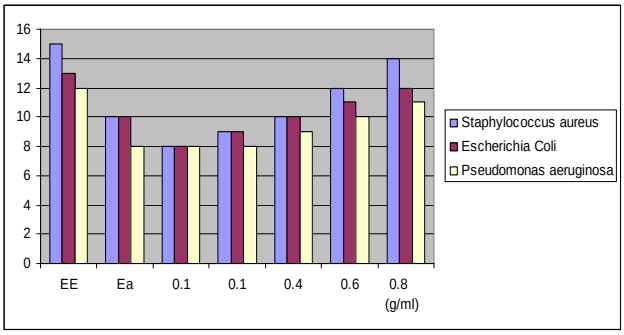

Fig.1: Sensitivity pattern of pseudomonas aeruginsoa, staphylococcus Aureus, Escherichia coli to ethanol extract of Garcinina Kola seed. (4g/40ml).

EE = Ethanol Extract
Ea = Ethanol Alone

Table1: The in-vitro antibacterial sensitivity crude aqueous extract (4g/40ml) of Garcina kola seed on test organism used showed no zone of inhibition at varying diluted concentration on the test organisms used which include, staphylococcus aureus, Escherichia coli and pseudomonas aeruginosa.

Table 1: The in-vitro antibacterial sensitivity crude aqueous extract (4g/40ml) of Garcina kola seed on test organism used.

Test Organisms	Size of inhibition of zone (mm)					
	AE	0.1	0.2	0.4	0.6	0.8 (g/ml)
Staphylococcus aureus	R	R	R	R	R	R
Escherichia Coli	R	R	R	R	R	R
Pseudomonas aeruginosa	R	R	R	R	R	R

R = Resistant
AE = Aqueous Extract

Table 2: In-vitro antibacterial sensitive of ethanol extract (40g/80ml) of Garcinia kola seed showed zone of inhibition as followed; ethanol extract showed on staphylococcus aureus 16mm, Escherichia coli had 13mm and pseudomonas had 12mm at different diluted concentration of 0.1gml, 0.2g/ml 0.4g/ml, 0.6g/ml and 0.8g/ml; staphylococcus aureus measured 9mm, 10mm, 13mm, 14mm, 15mm, Escherichia coli measured 8mm, 8mm, 9mm, 10mm and12mm and pseudomonas aeruginosa measured 7mm, 8mm, 8mm, 10mm, 11mm, and 12mm, respectively.

Table 2: In-vitro antibacterial sensitive of ethanol extract(40g/80ml) of Garcinia kola seed

Test Organisms	Size of inhibition of zone (mm)					
	EE	0.1	0.2	0.4	0.6	0.8
Staphylococcus aureus	16	9	10	13	14	15
Escherichia Coli	13	8	8	9	10	12
Pseudomonas aeruginosa	12	7	8	8	10	11

EE = Ethanol Extract

Table 3: In-vitro antibacterial sensitivity of Aqueous extract (40g/8ml) of Garcinia kola seed showed the zones of inhibition, the aqueous extract and diluted concentrations were all resistant to staphylococcus aureus, Escherichia Coli and pseudomonas aeruginosa.

Table 3: The in-vitro antibacterial sensitivity aqueous extract (40g/80ml) of Garcina kola seed

Test Organisms	Size of inhibition of zone (mm)					
	AE	0.1	0.2	0.4	0.6	0.8 (g/ml)
Staphylococcus aureus	R	R	R	R	R	R
Escherichia Coli	R	R	R	R	R	R
Pseudomonas aeruginosa	R	R	R	R	R	R

R = Resistant
AE = Aqueous Extract

IV. Discussion

This study revealed that crude ethanol Garcinia kola seed extract possess in-vitro anti-bacterial activities at varying concentrations which were sensitive to all the selected bacterial isolates, with staphylococcus aureus having the widest clear zones of inhibition followed by Escherichia coli, while pseudomonas aeruginosa had the smallest clear zone of inhibition, the crude aqueous extract at varying concentration against the selected clinical isolates were resistance to all the isolates. This is in conformity with findings as reported by [8] .that ethanol is the best solvent for the extraction of most plant active principles of medicinal properties.

The ethanolic extract of Garcinia kola seed extract showed more clear zones of inhibition than ethanol alone used as control, this shows that the seeds Garcinia kola possess many medicinal properties which include anti-inflammatory, anti-bacterial, antiviral, antidiabetic, purgative and antihepatotoxic effect as reported by Anegbeh *et al.*, 2006 [2]

The antibacterial activities at varying concentration showed different clear zone of inhibition with concentration of the neat showing the widest zones of inhibition followed by 0.8g/ml, 0.6g/ml, 0.4g/ml, 0.2g/ml and 0.1g/ml respectively. This shows that Garcinia kola seed extract is more effective at higher concentration.

The ethanolic extract of Garcinia kola seed (Bitter kola) were effective on staphylococcus aureus, Escherichia coli and pseudomonas aerugmosa which are Gram positive and Gram negative organisms, this shows that Garcinia kola can be used in treatment of Gram positive and Gram negative related disease.

The ethanolic extract of Garcinia kola seed (bitter kola) showed more zone of inhibition on staphylococcus aureus (Gram positive) and less zone of inhibition on Escherichia coli and pseudomonas aeruginosa (Gram Negative). This also shows that Garcinia kola is more effective on Gram positive bacteria than Gram negative bacteria.

V. Conclusion

Garcinia kola seed is sold commercially, so from this study, it has been proven that it should be used as an antimicrobial agent killing the most recalcitrant organism (Pseudomonas aeruginosa) Pharmaceutical companies are advised to take advantage of this opportunity.

This study showed that the invitro antibacterial activity of the seed extracts against the bacterial isolates at various treatment regimes may be attributed to the presence of phytochemical properties. Ethanol extract was found to have the significant activity against the clinical isolates in this study. This may be due to the fact that ethanol was found to be the best solvent for the extraction of active principles of medicinal importance in plants. So it is advised to take ethanol extract of Garcinia kola at various treatment regime.

Since aqueous extract was found to be resistance against the clinical isolates in this study, this may be due to the fact water is poor solvent for the extraction of active properties of medicinal plant. So taking aqueous extract of Garcinia kola may not give the require treatment.

VI. Recommendation

Therefore, it is recommended that further studies be carried out, to find out whether Garcinia kola can be remedy for Ebola virus and HIV virus because of its antiviral properties.

It should also be recommended that ethanolic extract should be used in treatment of staphylococcus, Escherichia coli and pseudomonas aeruginosa related diseases. The seeds should be chew as an aphrodisiac or used as remedies for dysentery, chest cold, liver disorder, hepatitis, diarrhea and laryngitis. It is also recommended in the treatment of stomach ache and gastritis.

REFERENCES

[1] M.I.E Ebomoyi, V.I. Iyawe. "Peak expiratory flow rate (PEFR) in young adult Nigerians following ingesaon of Garcinia kola (Heckel) Seeds"African Journal of Biomedical Research. 2000: 3,187-189.

[2] P.O., Anegbeh, C. Inika, C. Nkwika. "Enhancing germination of bitter kola (Garcinia kola) Heckel, Prospects for Agroforestry former in the Niger Delta". Science African 2006 (1), 25-29.

[3] E. Nzegbule, R. Mbakwe "Effect of pre sowing and incubation treatment of germination of Garcinia kola (Heckel) Seed Frurita". 2001:54, 437-442.

[4] D.E Okwu. "Phytochemical, vitamins and mineral contents of two Nigeria medicinal plant international" Journal of Molecular Medical and Advance Science. 2005. 1(4), 375-381.

[5] H. Manimi. M. Kinoshita, M. Sugiura. "Antioxidant xanthones from Garcinia subelliptica" Phytochemistry. 1994: 14, 533-629.

[6] M.M Iwu. " Food for medicine in: Dietary plants and masticatoires as sources of biologically active substances" University of Ife, Nigeria Ife Press. 1989:303-310.

[7] M.M Iwu. Handbook of African Medicinal plants. CRC Press incorporated. 1993;223-224.

[8] C.C. Ogueke, J.N. Ogbulie, H.O. Nsoku.. "Antimicrobial properties and preliminary phyto-chemical analysis of ethanolic extracts of Aistonia bonnie". Nigerian Science Microbiology.2006; 20(2), 896-899.

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- **Nwagu C.**, and Adegoke O. A,(2014) Malaria Parasitaemia and Some Haematological Parameters of In-Mate in Orphanage Home in Owerri Metropolis. *International Journal of Epidemiology & Infection* **2**(1):16-19
- **Mbata A. C.**, Adegoke O. A., **Nwagu C**., Wali A. N.,(2014) Studies on the Bacteria Burden in Garri Openly Sold in Port Harcourt Markets. *International Journal of Epidemiology & Infection* , **2**(4):75-79

CURRENT RESEARCH WORK

HLA Patterns And Quality Of Stem Cells From Cord Blood In Port Harcourt, South Nigeria.
this research work is aimed at

1. Studying the patterns of HLA A, B,C,DR,DQ and DP in cord blood using SSP-PCR
2. Assessing the quality of stem cells from these cord blood by measuring CD34 using flow cytometry .

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- **Mbata Christian A**, Adegoke Adebayo,Nwagu Chinyere, Nyeso Wisdom A Some Haematological Parameters in Diabetic Patients in Port Harcourt Nigeria. Volume 3, Issue 2, Feb. 2015

• **Mbata Christian A**, Nwagu Chinyere, Adegoke O.Adebayo." Comparative Analysis of Microscopy and Rapid Diagnostic Test (RDT) for the Laboratory Diagnosis of Malaria among Pregnant Women Attending Braithwaite Memorial Specialist Hospital, Port Harcourt" JMSCR Volume 3 Issue 1 January 2015

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