wjpmr, 2018,4(8), 254-259

SJIF Impact Factor: 4.639

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

EVALUATION OF THE ANTIMICROBIAL ACTIVITIES OF ETHANOL LEAF EXTRACT OF *Garcinia Kola (Heckel)* ON SOME PATHOGENIC MICROORGANISMS

Akeem A. Agboke* and Odudu O. Etukudo

Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Uyo, Uyo Nigeria.

***Corresponding Author: Akeem A. Agboke**

Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Uyo, Uyo Nigeria.

Article Received on 18/06/2018 **Article Revised on 09/07/2018** **Article Accepted on 30/07/2018**

ABSTRACT

This study was designed to investigate the antibacterial and antifungal activity of the ethanol extract of *Garcinia kola* leaf. Microorganisms used in the study were Pathogenic, *Staphylococcus aureus, Escherichia coli, Bacillus substilis, Pseudomonas aeruginosa and Candida albicans.* The result of the sensitivity test showed that at a concentration of 100 mg/ml of the extract had a higher activity against *Staphylococcus aureus* with inhibition zone of 30mm and *Bacillus substilis* inhibition zone of 20mm, whereas no activity against *Escherichia coli, Pseudomonas aeruginosa and Candida albicans* and with no zone of inhibition. At a concentration of 50 mg/ml the crude extract showed activity against *Staphylococcus aureus* with inhibition zone of 20mm and *Bacillus substilis* with inhibition zone of 15mm. At a concentration of 25 mg/ml, the crude extract showed activity against *Staphylococcus aureus* with inhibition zone of 12mm and *Bacillus substilis* with inhibition zone of 8mm. At concentrations of 12.5 mg/ml and 6.25 mg/ml, there was no activity against any microorganism used in the study. The result of minimum inhibition testing showed the MIC of crude extract against *Staphylococcus aureus* and *Bacillus substilis* to be 25 mg/ml, while other microorganisms did not have MIC. The result of minimum Bactericidal Concentration (after 48hours) of the crude leaf extract showed the MBC of the crude extract against *S. aureus* 25mg/ml and 50 mg/ml against *B. substilis.* This research confirmed the antimicrobial activity of the leaf extract of *Garcinia* kola against some pathogenic gram positive bacteria more potent than Azithromycin at concentration of 1500mg/ml that had no effect on the *S. aureus* and *B. Subtilis*. It is also confirmed that the crude extract has no activity against gram negative bacteria and *Candida Albicans.*

KEYWORDS: Antibacterial, Antifungal, Pathogenic, Minimum, Inhibition, Bactericidal.

INTRODUCTION

Microorganism have adapted to inhabit almost every corner of the world. They live in the oceans, and lakes, where they provide a valuable food source for larger organisms. They live on land where they may cause decay of organic matter, thus recycling valuable nutrients. Many even live in larger organisms where they may help hinder them (SparkNotes Editors). Humans have several reasons to be interested in the study of microorganisms. Many organisms cause diseases in humans. Bacteria and fungi can be parasites of human, causing anything from food poisoning to respiratory tract infection.

When microorganisms successfully invade the body and cause damage to tissues, infection is said to have occurred. Consequently, diseases produced by microorganisms are called infectious diseases. Infection may take place by;

- Physical contact with a diseased person.
- Droplet infection.
- Dust-borne infection.
- Contact with contaminated articles.
- Hand infection.
- Arthropod vectors.

Different factors influence infection. The ability of a microorganism to invade the host, establish itself in the tissues, and harm the host is called VIRULENCE.

Factors Affecting Virulence Include;

- Bacterial toxins (Exotoxins and Endotoxins), Enzymes; Hyaluronidase, Collagenase, Lecithinase, coagulase, Haemolysins, Leucocidins.
- The larger the number of organisms, the greater the chance of infection occurring.
- Routeof entry (e.g Typhoid through the alimentary canal, Gonorrhoea through genito-urinary tract and diphtheria through the respiratory tract) influences infection.
- Resistance of the host (prevention of entry, phagocytosis; fixed (macrophages) or wandering (Neutrophils) also influences infection.

Due to the ability of microorganisms to cause diseases and even death in humans, several antimicrobial agents have been discovered. These agents are of various origin such as natural, semi-synthetic or synthetic. However, the widespread and indiscriminate use of these agents have brought about the incidence of microorganism resistance, which have led to research on newer and more potent antimicrobial agent.

The universal role of plants in the treatment of disease is exemplified by their employment in all the major systems of medicine.

There is a great wealth of knowledge concerning the medicinal and other properties of plants that is transmitted from generation to generation by tribal societies.

Now monographs on crude plant preparations have made their way in modern pharmacopoeia. The use of modern isolation techniques means that new plant drugs usually find their way into medicine as purified substances rather than in the form of galenical preparations.

Over few decades, the role of medicinal plants as primary tool in preservation of health and management of diseases has been realized with great concern. This result mainly from the use of synthetic drug molecules that produce harmful side effects, which are comparatively minimal in drugs of plant origin (Ajoy *et al*., 2011).

Current estimates, suggest that in many developing countries, a large proportion of population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs. Although, modern medicine may be available in these countries, herbal medicines (phytomedicines) have often maintained popularity for historical and cultural reasons. Some of the drugs used today have originated from medicinal plants (Ajoy *et al*., 2011). The medicinal properties of these plants are attributed mainly to the presence of flavonoids, but they may also be influenced by other organic and inorganic compounds such as alkaloids, tannins, saponins, phenols etc. (Prabha *et al*., 2011).

Bitter kola botanically known as "*Garcinia kola*" is a herbaceous perennial medicinal plant grown in tropical rain forest in Central and West Africa (Uko *et al*., 2001; Okolle *et al*., 2009) and more predominantly in rainforest belt of Southern Nigeria (Agada and Braide, 2009). The tree is usually cultivated within villages in Southern Nigeria and grows to a height of about 12-14 m high. It has been referred to asa "wonder plant" because almost every part of it has been found to be of medicinal importance. The seed is also known as false fruit unlike kola nut (*Cola nitida*) which is known as "true kola". It is commonly called "Akuilu" in Igbo land, "Namijingoro" in Hausa land, "Orogbo" in Yoruba, and "Efiat" in Ibibio land of Nigeria. It produces characteristic reddish, yellowish or orange coloured fruit with seeds covered with skimoor husk.

Bitter kola *"Garcinia kola"* has been identified as potent antibiotics which could be effective in the treatment of many diseases such as high fever, jaundice and as purgative (Iwu *et al*., 1990). The seed is masticatory and is used to relieve and prevent chest cold and cough and can as well be used to treat headache (Ayensu, 1978). Bitter kola *"Garcinia kola"* could serve as raw material for pharmaceutical industries (Iwu *et al*., 1990). The medicinal use of the plant′s leaves and root in the management and treatment of diseases have been an age long practice (Sofowara, 1982). The plant exhibits very potent pharmacological activities such as antioxidant, antibacterial, antiviral, antifungal and anti-inflammatory properties (Adegboye *et al*., 2008.).

During recent years, considerable work has been done to investigate the pharmacological actions of the roots and seeds of *Garcinia kola*, while other morphological parts remain unexplored.

Actually, a lot of work has been done on the seed but limited research effort has been targeted to the leaf, even the bark.

Hence, the objective of this study is to evaluate the antimicrobial activity of the ethanolic leaf extract of *Garcinia kola*.

Anti-Oxidative Stress (Antioxidant) Effects

It was discovered that kolaviron from *Garcinia kola* at 200mg/kg body weight significantly reduced a tetra-butyl hydro peroxide induced in 2-amino-adiposemialdehyde (2-AAS) a maker of protein oxidation in both plasma and liver, hence decreasing oxidative damage to DNA in the liver. (Farombi *et al*., 2004). In another study carried out by Farombi and another group of researchers, kolaviron from *Garcinia kola* (100mg/kg body weight for 1week) protected rat liver cells against H_2O_2 induced DNA strand breaking, oxidized purine (Formamidopyrimidine glycosylase (FPG) and pyrimidine (endonuclease III (ENDO III) sites bases, sensitive sites both in rat liver and human lymphocytes, and Fe3+/EDTA/ ascorbate-induced malondialdehyde formation and protein oxidation. Gamma glutamyl semialdehyde (GGS) and 2- amino adiposemialdehyde (2-AAS) were used as biomarkers of oxidative damage to protein. They suggested that kolaviron exhibits protective effects against oxidative damage to molecular targets through the scavenging of free radicals or iron binding (USSDA 2007).

Kolaviron may therefore be relevant in the chemoprevention of oxidants induced genotoxicity and possibly human carcinogenesis. **(**Farombi *et al*., 2004**)** The *Garcinia* fruit also contains xanthones, which inhibit pre-neoplastic lesions in mammary and colon cancer. The xanthones may also induce apoptosis in mouth,

leukemia, breast, gastric, and lung cancer cell lines in vitro (Mazzio and Soliman, 2009). Another study that found that supplementation with *Garcinia kola* can reduce oxidative damage is Yonei *et al*, (2008).

Antibiotic Effects

Aqueous and alcohol extracts of *Garcinia kola* was found to inhibit organisms like *staphylococcus aureus*, *klebsiella pneumonia*, beta-hemolytic streptococci, *Escherichia coli* and *Neisseria gonorrhea* (Ebana *et al*., 1991). *Garcinia kola* extract was also demonstrated to be effective against *staphylococcus aureus*, *streptococcus pneumonia*, and hemophilius influenza at a minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) (Akochere *et al*., 2002).Other researchers that observed antibacterial activities are Hussain *et al*, (1982) and Han *et al*., (2005) who observed that *Garcinia kola* root extract is also effective against methicillin resistant *staphylococcus aureus* (MRSA) and vacomycin resistant *Escherishia coli* (VRE). Others are Adeboye *et al*., 2008 and Sibanda and Okoh., 2008.

MATERIALS AND METHODOLOGY

Materials

Measuring cylinders, funnels, water bath, beakers, conical flasks, filter paper, sample bottles, ethanol, leaves of *Garcinia kola*, conical flasks, vials, Measuring cylinders (100ml, 20ml), Autoclave (Ketan SAS2^{R}), Incubator (Electrothermal incubator, Model DNP^{R}), Sterile water, Meuller Hinton Agar, Nutrient broth, sterile petri dishes, flame source, test tubes, pipette (2ml, 5ml), stainless steel borer, isolates of *S. aureus*, *P. aeruginosa*, *E. coli*, *B. substilis* already in stock from microbiology laboratory in University Of Uyo Teaching Hospital, Uyo, Akwa Ibom State.

Reagents

Distilled water, 1% Hydrochloric Acid, Dragendorff's reagent, Ferric chloride, Lead acetate, glacial acetic acid, Concentrated tetraoxosulphate vi acid (H_2SO_4), Ethanol, acetic acid, acetic anhydride, 10% ammonia solution, chloroform, ethyl acetate, phosphate buffer, Asbestos disc,1% cholesterol, Ether, Oxylol, 7% blood nutrient, Benzene, 10% Ammonia, Azithromycin 1500mg, Nystatin.

Plant collection, Preparation and Extraction

The fresh leaves of Garcinia kola was procured in the month of October, 2017 from the village Ikot Ntuen Nsit, Nsit Ibom Local Government Area, Akwa Ibom State and was identified by Mrs Emannuela Godwin Udoma (The curator of the department of pharmacognosy herbarium, University of Uyo, Uyo). The fresh leaves were cleaned by passing under running water and dried in a sunny environment (under shade) until dried, they were then reduced with the aid of a mortar and pestle to small sizes. 173.3kg of the powdered leaves were put in an extraction jar, 50% ethanol was added, the extraction jar was sealed and allowed to stand at room temperature for 72 hours for complete extraction to occur while intermittently agitating the flask throughout the period of extraction.

After 72 hours, the contents of the extraction jar was filtered, the filtrate was transferred to a conical flask and concentrated to dryness in a water bath at 40^{o}C. The extract was then weighed into an already weighed beaker and stored until when needed. Weight of extract was 105g.

Phytochemical screening of *G. Kola* Ethanol leaf extract

The crude Ethanol extract was used to test for Alkaloids, Tannins, Saponnins, Cardiac glycosides, Flavonoids, and Anthraquinones. Using standard methods, Iwu, Trease and Evans).

Microbiological Assay of Extract

Preparation of culture media

Nutrient Broth Liquid Media

1.3g nutrient broth powder was added to 100ml of distilled water and mixed well, it was then heated for complete dissolution.

Meuller Hinton Agar

3.8g of Meuller Hinton agar was weighed and added to 100mL of distilled water in an Erlenmeyer flask, the mixture was then heated for complete dissolution. It was then autoclaved at 121^{o}C for 15minutes.

Preparation of Microbial culture

The reference bacterial and yeast strains used in this research work were obtained from the Microbiology laboratory, University of Uyo Teaching Hospital, Uyo, Akwa Ibom State. They include *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphyloccocus aureus*, *Bacillus substillis*, and *candida albicans.*

Sensitivity testing of the antimicrobial activity of *G. kola* leaf crude extract by agar well diffusion

Using a sterile Pasteur pipette, 1ml of the inoculums was aseptically transferred into the labelled petri-dish and the lid covered quickly, 15mL of molten meuller Hinton agar was poured over the inoculum in the dish. The dish was gently swirled to allow proper mixing and then allowed to cool and solidify.

Wells measuring about 8mm in diameter were cut out under aseptic conditions using a sterile stainless borer and each well was filled with 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL of each fraction. The dis was allowed to stand to allow for proper diffusion before further incubation in an upright position at 37^{o}C for 24hours in the case of bacteria and 27^{o}C for 24hours in the case of yeast, a negative control using sterile distilled water and positive control using Azithromycin disc (for bacteria) and nystatin (for yeast) was used. The different zones of inhibition were measured (Sarin *et al*., 2012).

Determination of the minimum inhibitory concentration of the crude extract of *G. kola*

The aim of this experiment is to determine the minimum inhibitory concentration of crude extract using varying concentrations.

Two-fold serial dilution was carried out using the crude extract to obtain concentration of 100 mg/mL, 50 mg/mL, 25 mg/mL and 12.5 mg/mL in tubes containing nutrient broth. Using a Pasteur pipette, about 0.01ml of the overnight broth was inoculated into the tubes, the tubes were then sealed and inoculated at 37^{o}C for 24hours (for bacteria) and 27^{o}C for 24hours for yeast. The tubes were then checked for presence or absence of growth and the results were recorded.

RESULTS

Phytochemical screening

Table 1: The result of the phytochemical screening of the ethanol leaf extract of *Garcinia kola.*

Akaloids	
Dragendorf's Reagent	_
Mayer's reagent	_
Tannins	
Ferric chloride test	+
Saponins	
Frothing test	_
Red blood haemolysis	_
Fehling's A+B	_
Cardiac glycosides	
Salkowski test	+
Keller Killiani test	++
Lierbermans test	+
Flavonoids	
Magnesium metal test	+
Sodium hydroxide test	+
Ammonium hydroxide test	+
Anthraquinones	
Combined anthraquinones. (Benzene used)	+
Free anthraquinone (benzene used)	_

Key: (+) -Trace, (++) - Moderately present, **(-) -** Absent

Results of Sensitivity Testing

Table 2: Extract against Staphylococcus *aureus.*

Concentrations	Results
100mg/mL	_
50mg/mL	_
25mg/mL	-
12.5mg/mL	+

Key: (+) Presence of growth, **(-) -** Absence of growth

Table 3: Extract against Baccilus substilis.

Concentrations	Results
100mg/mL	_
50mg/mL	_
25mg/mL	+
12.5mg/mL	+

Key: (+) Presence of growth, **(-) -** Absence of growth

Table 4: Extract against *Pseudomonas aeruginosa.*

Concentrations	Results
100mg/mL	+
50mg/mL	+

Key: (+) - Presence of growth

Table 5: Extract against *Escherichia coli.*

Concentrations	Results
100mg/mL	+
50mg/mL	+

Key: (+) - Presence of growth

Table 6: Candida albicans.

Concentrations	Results
100mg/mL	+
50mg/mL	+

Key: (+) - Presence of growth

Minimum Inhibitory Concentration of the Crude Extract

Table 7: Extract against *Staphylococcus aureus.*

Concentration(mg/ml)	Zone of inhibition(mm)			Mean ± SEM
	I	II	III	
100mg/ml	30.00	30.10	29.00	30mm ±0.4mm
50mg/ml	20.00	19.00	20.20	20mm ± 0.4mm
25mg/ml	12.00	12.00	12.00	12mm ± 0.0mm
12.5mg/ml	0.00	0.00	0.00	0.00
Azithromycin1500mg	0.00	0.00	0.00	0.00
Distilled water	0.00	0.00	0.00	0.00

Table 8: Extract against *Bacillus substilis.*

Concentration(mg/mL)	Zone of inhibition(mm)			Mean ± SEM
	I	II	III	
100mg/mL	20.00	21.00	19.80	20mm ± 0.42mm
50mg/mL	14.90	15.00	15.20	15mm ± 0.12mm
25mg/mL	8.00	8.00	8.00	8mm ± 0mm
12.5mg/mL	0.00	0.00	0.00	0.00
Azithromycin 1500mg	0.00	0.00	0.00	0.00
Distilled water	0.00	0.00	0.00	0.00

Minimum Bactericidal Concentration Test

Table 12: Crude Extract against *Staphylococcus aureus.*

Concentrations	Results
100mg/mL	_
50mg/mL	-
25mg/mL	+
12.5mg/mL	+

Key: (+) Presence of growth, **(-) -** Absence of growth

Table 13: Extract against *Bacillus substilis.*

Concentrations	Results
100mg/mL	_
50mg/mL	+
25mg/mL	+
12.5mg/mL	+

Key: (+) - Presence of growth, **(-) -** Absence of growth

DISCUSSION

The result of the preliminary phytochemical screening and microbiological assay are depicted on the respective tables.

The preliminary phytochemical test was qualitative in nature and from the investigation, it was observed that the ethanol extract of *Garcinia kola* leaf contains secondary metabolites such as alkaloids, tannins, flavonoids, cardiac glycosides, saponins and anthraquinones.

From the antimicrobial sensitivity investigation, it was observed that the ethanol (crude) extract of *G. kola* leaf exhibited considerable activity against *Staphylococcus aureus* and *Bacillus substilis*, at concentrations of 100 mg/ml, 50 mg/ml and 25 mg/ml. However, it showed no activity against *Pseudomonas aeruginosa, Escherichia coli* and *Candida albicans*.

The fact that *G. kola* ethanolic leaf extract possesses antibacterial activity was stated by Ebana *et al*., 1991. This antibacterial activity is due to the following antibacterial agents; kolaviron, xanthones (Farombi *et al*., 2004), poly-iso-phenyl benzophenone (kolanone), and ethyl acetate (Macluhan, R; 1995). The inactivity exhibited by *G. kola* leaves against *E. coli, P. aeruginosa* and *C. albicans* may perhaps be due to the absence of inhibitory alkaloids against these organisms. This is because alkaloids have been claimed to be responsible for antimicrobial effect (walter and Nowaki, 1978). This is similar to the report of Burger (1990), who showed that no active substance exhibited its maximum activity under laboratory experimental conditions. Therefore, activity may be recorded if greater concentrations are used. In addition, ingredients of the media (Bevallius and Zacharias; 1971), P^H size and inoculum (stokes and Ridway, 1980) may be attributed to the inactivity of the extract.

The zones of inhibition of the ethanol extract of *Garcinia kola* leaf (100mg/ml) are as follows; *S. aureus* (30mm ± 0.4mm), *B. subtilis* (20mm ± 0.42mm), 50mg/ml; *S. aureus* (20mm ± 0.4mm), and *B. substilis* (15mm ± 0.12mm). From the results obtained, Azithromycin disc (1500mg) had no zone of inhition on *S.aureus*, and *B.substilis*, this might be due to resistant strain of the microorganism. Azithromycin (1500mg), had an inhibitory zone of 27mm on *P. aeruginosa*, but the crude extract had no zone of inhibition on *P. aeruginosa*. Azithromycin (1500mg) gave 30mm as zone of inhibition on *E.coli*, but the crude extract had no zone of inhibition on *E.coli.*

It was observed that the minimum inhibitory concentration of the crude extract against *S. aureus was* 50 mg/ml and the same concentration was for *B. substilis*. The minimum bactericidal concentration testing after 48hours showed a concentration of 50mg/ml for *S. aureus* and MBC concentration of 100mg/ml was obtained for *B. substilis.* This shows that *Bacillus substilis* is less susceptible to the crude extract than *S. aureus.*

CONCLUSION

These findings confirmed that the leaf of *Garcinia kola* contains antimicrobial principles that can be further developed for the therapy of *S. aureus* and *B. substilis* infections and has thus suggested a new pathway in elucidating potent antimicrobial agents from *Garcinia kola* leaf. Also, this investigation has shown the minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC) of *Garcinia kola* leaf against *S. aureus* and *B. substilis* which is very low compare to the Azithromycin disc of 1500mg/ml this is very important when developing lead compounds.

CONFLICT OF INTEREST

There is no conflict of interest in the process of this research work.

ACKNOWLEDGEMENT

We acknowledged Mrs Emannuela Godwin Udoma (The curator of the department of pharmacognosy herbarium, University of Uyo, Uyo for her contribution in authenticating the *Garcinia* kola leaf.

REFERENCES

1. Adegboye MF, Akinpelu DA, Okoh A The bioactive and phytochemical, 2008.
2. properties of *Garcinia kola* (Heckel) seed extract on some pathogens. *African Journal of Biotechnology*, 7(21): 3934-3938.
3. Agada, P.O and V.B Braide, Effects of dietary *Garcinia kola* seed on selected serum electrolytes and trace metals in male albino rats. Nig. J. Physiol. Sc., 2009; 24: 53-57.
4. Ajoy, K.G; B. Souvar. B. Halder and R. B. Ni-shith, An overview on different variety of Musa species; importance and its enormous pharmacological action. J.Pharm. Herb. Formul, 2011; 1(2): 1-11.
5. Ayensu, E.S; Medicinal plants of West Africa. Reference published incorporated, Algonac MI, USA, 1978; 162.
6. Akochere J. F, Ndip R.N, Chenwi E.B, Ndip L.M, Njock T.E, Anang D.N, Antibacterial effects *Zingiber officianel* and *Garcinia kola* on Respiratory tract pathogens. *East African Medical Journal*, 2002; 79(11): 588-592.
7. Bevallius, B. and Zacharias, N. Applied Microbiology in Clinical Bacteriology 5thEdition, 1971; 72-79.
8. Ebana RU, Madunagu BE, Ekpe ED, Otung IN Microbiological exploitation of cardiac glycosides and alkaloids from *Garcinia kola*, Borreria ocymoides, Kola nitida and Citrus auratifolia.*Journal of Applied Bacteriology*, 1991; 71(5): 398-401.
9. Farombi E. O, Adepoju B. F, Ola-Davies O. E, Emerole G. O Chemoprevention of Aflatoxin B1 – Induced genotoxicity and hepatic oxidative damage in rats by kolaviron, a natural bioflavonoid of Garcinia kola seed. *European Journal of Cancer Prevention*, 2005; 14(3): 207-214.
10. Farombi E. O., Hansen M., Raven-Haren G, Moller P., Dragsted L.O. Commonly consumed and Naturally Dietary Substance Affect Biomarkers of Oxidative Stress and DNA Damage in Healthy Rats. *Food Chemical Toxicology*, 2004; 42(8): 1315-541.
11. Hussain, R. A., A. G. Owegby, P. Parimoo,P. G. Waterman Kolanone, a novel polyisoprenylated benzophenone with antimicrobial properties from the fruit of *Garcinia kola*. *Planta Medica*, 1982; (44): 78-81.
12. Han Q.B, Lee S.E, Qiao C.F, He ZD, Song JZ, Sun HD, Xu Hx. Complete NRM assignment of the antibacterial biflavanoid GB1 from *Garcinia kola*. *Chemical Pharmacology Bulletin* (Tokyo), 2005; 53(8): 1034-1036.
13. Iwu, M.M; A.O. Agbako and M.S Tempesta, Antidiabetic and aldose reductase activities of biflavonones of *Garcinia kola* J. Pharm. Pharmacol, 1990; 42: 290-292.
14. Iwu M.M Ebola cure hope. BBC Health News; Thursday 5th August, 1999. Assessed from http://www.bbc.co.uk/2/hi/health/411030.stm
15. Macluhan, R., Antimicrobial activities of theconstituents of Garcinia kola seeds. Int. J. Pharmacog, 1995; 33(3): 25-30.
16. Okelle, J.N; G.H. Fansi and F.M. Lombi, Banana entomological research in Cameroon; howfar and what next? Afri. J. Plant Sci. Biotech, 2009; 3(1): 1-19.
17. Prabha, P; T. Karapagam, B. Valralakshine and A. S. Pack iavathy, Indigenous antiulcer activity of *Musa sapientum* on peptic ulcer. Pharmacog. Res, 2011; 3: 232-238.
18. Sibinda T. and Okoh A. Invitro evaluation of interactions between acetone extract of *Garcinia kola* and some antibiotics. *African Journal of Biotechnology*, 2008; 7(11): 1672-1673.
19. Sofowara, E.A. Medicinal plants and traditional Medicines in Africa. John Wiley and Ltd; Nigeria, 1982; 64-79.
20. Uko, O.J; A. Usman and A.M. Ataja, Some biological activities of *Garcinia kola* in growing rats. Veterinaski. Arhiv, 2001; 71: 287-297.
21. USSDA *Garcinia kola*. Heckel. Horse Radish tree plant profile. The plant Database. http://plants.usda.gov/java/profile? Symbol=Mool. National plant data center, natural resources conservation service (NRCS), United State Department of Agriculture (USDA), Baton Rouge, Lousian, USA. Accessed 13 August, 2007.
22. Waters A. E; Contente-cuomo, T; Buchhagen J. et al "Multi-drug resistant *staph aureus in USA* meat and poultry". *Clin. Infect. Dis.,* 2011; *52(10): 1227-1230.*
23. Yonei Y, Takahashi Y, Hibino S, Watanabe M, Yoshioka T Effects on the human body of a dietary supplement containing L-carnitine and *Garcinia cambogia* extract: a study using double-blind tests. *Journal of Clinical Biochemistry and Nutrition*, 2008; 42(2): 89-103.