American Journal of
Biomedical Science & Research

www.biomedgrid.com
ISSN: 2642-1747

Research Article

In Vitro Antibacterial Activity of *Garcinia Kola* Aqueous Stem Extract on Bacterial Isolated from Patient Visiting a Tertiary Health Facility in South-South Nigeria

Iyevhobu KO[1,2]*, Edo EO[3], Airefetalor A[1,2], Jabbo AA[3] and Malgwi JM[4]

[1]*Department of Public Health, National Open University of Nigeria, Uromi, Nigeria*

[2]*Lassa Fever Enable Study CEPI/ISTH Irrua, Nigeria*

[3]*National Ear Care Centre, Kaduna, Nigeria*

[4]*Department of Biochemistry, Modibo Adama University, Nigeria*

***Corresponding author:** Iyevhobu KO, Department of Public Health, National Open University of Nigeria, Uromi Community Study Centre, Lassa Fever Enable Study CEPI/ISTH Irrua, Edo State, Nigeria.

To Cite This Article: *Iyevhobu KO, Edo EO, Airefetalor A, Jabbo AA, Malgwi JM. In Vitro Antibacterial Activity of Garcinia Kola Aqueous Stem Extract on Bacterial Isolated from Patient Visiting a Tertiary Health Facility in South-South Nigeria. Am J Biomed Sci & Res. 2022 - 17(1). AJBSR. MS.ID.002318.* DOI: 10.34297/AJBSR.2022.17.002318

Received: September 07, 2022; **Published:** September 21, 2022

Abstract

Resistance to treatment with antibiotics has promote herbal concern due to increasing failure of antibiotics in treatment of bacterial infections, it is therefore necessary to test the potency of herbal derivatives. The antimicrobial effect of *Garcinia kola* stem extract was investigated against *Escherichia coli, Pseudomonas aeruginosa, Streptococcus species, Proteus mirabilis, Staphylococcus aureus, Klebsiella pneumonia and Salmonella species* (Gram +ve and -ve bacteria, respectively) using disc-diffusion method (Kirby-Baur). Aqueous stem extracts of 25mg/ml, 125mg/ml and 250mg/ml showed considerable inhibitory activities against the isolates. Antibacterial activity indices (AL) with variable inhibition zones (IZ) to test bacteria isolates range from 10-16mm for *Escherichia coli*, 13-16mm for *Staphylococcus aureus*, 6-14mm for *Proteus mirabilis,* 7-20mm for *Klebsiella pneumonia*, 7-11mm for *Pseudomonas aeruginosa,* 6-12 for *Streptococcus species* and 9mm for *Salmonella species*. The *Garcinia kola* stem extract had Minimum Inhibitory Concentration (MIC) of 7.28mg/ml for *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumonia* respectively, *Streptococcus species* and *Proteus mirabilis* had MIC of 31.25mg/ml while *Pseudomonas aeruginosa* and *Salmonella species* had MIC of 62.25mg/ml respectively. The Minimum Bactericidal Concentration (MBC) in this study, showed that 1.92mg/ml minimum concentration of the aqueous extract have bactericidal activity for *Klebsiella pneumonia*, 7.28mg/ml for *Escherichia coli* and *Proteus mirabilis*, 15.68mg/ml for *Staphylococcus aureus*, 125mg/ml for *Pseudomonas aeruginosa,* 62.28mg/ml for *Salmonella species* and 62.28mg/ml for *Streptococcus species*. Phytochemical screening, revealed that, *Garcinia kola* stem extract contains Tanin, Resin, Flavonoid, Cardiac glycoside, Alkaloid, Resin, saponin and total absence of steroid. The present of bioactive metabolites in stem extract indicate its anti-microbial and therapeutic capabilities and supports folkloric claim that *Garcinia kola* is an effective tool used as herbal remedy and if purified, it could be employed in pharmaceutical formulations.

Keywords: Antibacterial, *Garcinia kola*, Aqueous, Extract, Bacterial

Introduction

The study of medicinal plants used in folklore remedies in the treatment of microbial infections have attracted the attention of many scientists as possible alternatives to the existing drugs to which many infectious microorganisms have become resistant. Presently, there are global problems of multiple antibiotics resistance as well as emergence of new and resurrection of previously eradicated diseases. Reports on ethnobotanical records indicate a consensus on the use of antimicrobially active medicinal plants to provide cheaper drugs. There is need to search for new and more potent antimicrobial compounds of natural origin to complement the existing synthetic antimicrobial drugs that are gradually becoming less potent against pathogenic

microorganisms. This study therefore focuses on the antimicrobial potency of *Garcinia kola* stem extract. *G. kola* belongs to the family *Guittiferae* and it is commonly called "*Orogbo*" in Yoruba language while the English name is bitter kola. The plant has been referred to as a "wonder plant" because every part of it has been found to be of medicinal importance [1]. *G. kola* is used in folklore remedies for the treatment of ailments such as liver disorders, hepatitis, diarrhoea laryngitis, bronchitis, and gonorrhoea [2,3]. The seed is masticatory and used to prevent and relieve colic, chest colds, cough and can as well be used to treat headache [4]. *Iwu* [2] reported the use of this plant for the treatment of jaundice, high fever, purgative by chewing stick of garcinia kola. The plant also found usefulness in the treatment of stomachache and gastritis [5]. The phytochemical compounds isolated from *G. kola* include oleoresin Onayade, et al. (1998), tannins, saponins, alkaloids, cardiac glycosides Ebana, et al. (1994). Other phytochemical compounds so far isolated from *G. kola* seeds are biflavonoids such as kolaflavone and 2-hydroxybi-flavonols [6-8]. The biflavanones are predominant compounds in *G. kola* and kola flavanones are major components of kolaviron [9]. *G. kola* is used in folklore remedies for the treatment of various infections caused by pathogens.

The use of herbs in the treatment of man and animal diseases has long been practiced before the advent of antibiotic. *Garcinia kola* seed medicinal properties has long been known, considered, and recommended by herbal traditional healers in the treatment of varieties of illness caused by bacterial infection. Its stem, root and leave concoction is also a remedy administered by oral rout. The pharmacological properties are undefined which result to investigation of the in vitro studies of the antibiotic activity to different pathogenic bacterial isolated to establish its antibiotic properties and determine the antibiotic spectrum to clarify the claims of traditional users of stem bark of *Garcinia kola* stem concoctions in treatment bacterial infections. This study therefore focused on the bioactive potentials of the extract from the bark plant on some microorganisms. This study is set to establish the antibacterial properties and activity of *Garcinia kola* stem extract on pathogenic bacteria isolates.

Materials and Methods

Source of *Garcinia kola Stem*

Fresh stem bark of *Garcinia kola* (bitter kola) was obtained from *Garcinia* kola tree from Ekpoma plant farm in Esan West Local Government Area, Edo state, Nigeria. Ekpoma is the headquarter of Esan West Local Government area of Edo State. It is located at latitude 6°45ᴵN and longitude 6°08ᴵE. It is moderately populated with the peoples's occupation being farming and trading. The main sources of water in the locality are rainfall and well. The well is augmented by irrigation scheme provided by the Government for public use. University is situating in this region. It is usually cold at night and very hot during the day. It also has undulating topography World Gazetter, et al. (2007).

Processing of *Garcinia kola Stem* Bark

The stem bark of *Garcinia kola* was cut into pieces and then oven dried at 40°C for 10 days before it was processed for extraction.

Grinding of *Garcinia kola* Stem Bark

The cut stem bark of *Garcinia kola* was grinded to powder using a blender and was kept in a sterile universal container for extraction.

Extraction of *Garcinia kola* Stem Bark

The stem bark of (*Garcinia kola*) was extracted using the following solvents: Cold water at room temperature, Hot water (80°C), 70% ethanol and Ether.

Water Extraction: This was performed in sterile universal bottle by adding 20ml of sterile distilled water to the different grams of the powdered of *Garcinia kola* as follows; 0.5g, 2.5g, and 5g to give a total concentration of the extract in mg/ml. and were kept in cool and dry place for 24 hours before heating to evaporate to obtain the required aquose extract for susceptibility testing.

70% Ethanol: This was obtained by diluting absolute Ethanol of (99.9%) with sterile distilled water at 7/3 dilution, i.e 7ml of ethanol to 3ml of diluents and was used to extract grinded *Garcinia kola* stem.

Ether Extract: This was obtained by obtaining 20ml of ether to the different grams of grinded *Garcinia kola* stem extract in a brown bottle.

Preparation of Dilution Extracts

The extract of *Garcinia kola* stem was placed into a sterile universal container and was diluted with 20ml of sterile distilled water, ethanol, and ether to obtain a final concentration of the extracts in mg/ml. It was allowed to stand for 24hourse and filtered. The filtrate was kept and evaporated for susceptibility activities of the extract.

Phytochemical Screening Test for the Extracts

This was done on the different method of extraction to ascertain the presence of bioactive compound in the cool water and 70% ethanol extract of *Garcinia kola.* The presence of Tannin, Resin, Alkaloid, Saponin, Cardiac glycosides, Flavoniod and Steroid will be determined with Trease Evans method [10].

Source of Bacterial Isolates

The different test organisms were isolated from urine, throat swabs, stool, sputum, and nasal swab from clinical samples of patient visiting Irrua Specialist Teaching Hospital (ISTH) located in Irrua Local Government Edo State and was analyzed using Centre

for Disease Research laboratory (CDR), for period of three months (December 2021-Febuary, 2022) located in Uwen Emuado Ekpoma. The test organisms isolated for the study include *Staphylococcus aureus, Klebsiella pneumonia, Streptococcus species*, *Proteus Mirabilis*, *Pseudomonas aureginosa, Escherichia coli and Salmonella species.*

Identification of Test Organisms

All isolates for this study were identified by their colonial morphology on the media which include Size, Shape, Elevation, Opacity, Edge, Colour, haemolysis and fermentation, Gram stain reaction, biochemical test characterization and sugar fermentation test.

Preparation of Test Organisms

The different organism isolated; *Staphylococcus aureus, Klebsiella pneumonia, Streptococcus species*, *Proteus species*, *Pseudomonas species, Escherichia coli and Salmonella species* were sub-cultured into peptone water for 8 hours before antibiogram extract testing.

Antimicrobial (Antibiogram) Activity Test

The antibacterial properties of the extracts were tested against each isolate comprising of both Gram-positive and Gram-negative organism using disc diffusion method as described by Kirby- Baur, et al. (1966).

Sensitivity Testing of *Garcinia Kola*

The sensitivity testing of the plant stem extract was determined using the disc diffusion method as described by Kirby-Bauer, et al. (1966). The antimicrobial disc was locally prepared by punching out disc 6mm in diameter from a good quality Whatman no 1 filter paper and was sterilized for 15 minutes with autoclave. The bacterial isolates were first inoculated in peptone water and incubated for 8 hours and was sub-cultured on nutrient agar, excess broth was tilled off and allowed to dry. Using a sterile forceps, the prepared sterile disc was picked and impregnated with the various extract with different concentration (25mg/ml, 125mg/ml, and 250mg/ml) in 0.01ml and the disc placed on the inoculated agar plate equidistant from each other alongside with two locally prepared antibiotic disc (ciprofloxacin and tetracylclin) as positive control and diluents as negative control. It was incubated at 37°C for 24 hours in an incubator and inhibition zone (IZ) was measured by using ruler calibrated in millimeters.

Minimum Inhibitory Concentration (MIC)

Tube dilution method by Cowan and Steel, et al. (1985). The minimum inhibitory concentration gave the lowest concentration of the aqueous extract that can inhibit the growth of the bacteria isolates. 0.5ml prepared nutrient broth was dropped into the test tube 2 to 10, 0.5ml of the extract was added to tube 1 and 2. Serial dilutions were made resulting to decreasing concentration of the aqueous extract. The extract in tube 3 was diluted until tube 9 from which 0.5ml was discarded. 0.5ml of the test bacteria isolates were added to tube 2 to tube 10. Tube1 which contain nutrient broth and organism serves as control tube [6]. The entire procedure was done for the bacteria isolates that were susceptible to the extracts with zone of inhibition above 10. The tubes were thoroughly mixed and incubated at 37°C for 24hours after which they were examined for visible growth which is seen as turbidity. The MICs reported as the lowest concentration of the *Garcinia kola* extracts that prevent visible growth [11-15].

Minimum Bactericidal Concentration (MBC)

The minimum bactericidal of *Garcinia kola* stem extract was determined with little modification, by obtaining samples in the MIC assay and Sub-cultured onto freshly prepared nutrient agar medium and incubated at 37°C for 48 hours. The MBC was taken as the lowest concentration of the extract that did not show any visible bacterial growth on the surface of the agar plate [11].

Results

Table 1: Phytochemical Screening Results of *Garcinia kola* (Bitter Kola) Aqueous Stem.

Phytochemicals	Extraction Type			
	Cold water	Hot water (80°C)	Ethanol (70%)	Ether
Flavonoid	_	+	+	+
Tanin	_	_	+	+
Resin	+	+	_	+
Cardiac glycoside	_	_	+	+
Saponin	+	_	_	_
Alkaloid	+	+	_	_
Steroid	_	_	_	_

Note*: Key: + = positive; - = Negative

From the aqueous stem extract of *Garcinia kola* (cold water, hot water, ethanol, and ether). Phytochemical screening shown the presence of Tanin, Flavonoid, Resin and Cardiac glycoside in ether aqueous extract and the presence of Alkaloid and Resin in cold and hot water aqueous extract with the presences Saponin in cold water extract only and total absence of Steroid in all the type of extraction used in this study (Table 1).

Aqueous stem extracts of *Garcinia kola* (minimum concentration of 25mg/ml to maximum concentration of 250mg/ml) antibacterial Activity Indices (AL) showed variable inhibition zone (IZ) to test bacteria isolates. The IZ of the various type of extraction for the different test bacteria isolates, had 10-16mm for *Escherichia coli*, 7-11mm for *Pseudomonas aeruginosa,* 6-12 for *Streptococcus species*, 7-13mm, for *Proteus mirabilis,* 13-16mm for *Staphylococcus aureus*, 7-20mm *Klebiesella pneumonia*, 6-14mm for, *Streptococcus spices* and 9mm for *Salmonella species*. The negative control (diluents used for extraction) that is, sterile distilled water and hot water, showed no IZ to the bacterial isolates while 70% Ethanol and ether showed 6-8mm IZ to the bacteria isolates. Ciprofloxacin [CPX-5µg/ml (Broad Spectrum Antibiotics)] and Tetracycline [TTC-30µg/ml (Narrow Spectrum Antibiotics)] was used as positive control CPX gave IZ for all the bacteria isolates ranging from 14-35mm while TTC gave IZ ranging from 9-23mm to the test bacteria isolates except *Salmonella species* with IZ of 9mm and *Pseudomonas aureginosa* with IZ of 8mm (Table 2).

Table 2: Antibacterial Activity of *Garcinia kola Stem* Extract on Bacteria Isolates.

Extraction	Test Organisms [Mean zone of Inhibition (mm)]						
	E. coli	***P. aeruginosa***	***P. mirabilis***	***S. aureus***	***Kleb. pneumoniae***	***Strept. spp***	***Salm. spp***
Cold water	Dec-13	07-Aug	0	14	10	6-9	0
Hot water (800C)	10	8-9	6-7	0	7	0	9
Ethanol (70%)	13 - 14	9	6-9	13 - 16	18-20	6-11	0
Ether	14-16	8-11	7-12	13 - 15	12-18	6-14	9
Control:							
+C (CPX)	35	25	25	36	35	23	21
+C (TTC)	18	8	23	28	16	31	9
-C (DL)	06-Jul	0	6	6-8	7	7	06-Aug

Note*: KEY: +C Positive control; -C Negative Control; CPX-Ciprofloxacin; TTC-Tetracyline; DL-Diluent and *Spp-species*.

The *Garcinia kola* stem extract Minimum Inhibitory Concentration (MIC) showed smallest concentration of the aqueous stem extract with minimum growth of the bacteria isolates when tested. The Bitter kola aqueous stem extracts had minimum inhibitory concentration of 7.28mg/ml for *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumonia* respectively, *Streptococcus species* and *Proteus mirabilis* had MIC of 31.25mg/ml while *Pseudomonas aeruginosa* and *Salmonella species* had MIC of 62.25mg/ml respectively (Table 3).

Table 3: Minimum Inhibitory Concentration (MIC) of Garcinia kola Stem Extract on Bacteria Isolates.

Test organisms	Concentration of Extracts in mg/ml (250-0.34)-MIC
Escherichia coli	7.82
Pseudomonas aeruginosa	62.2
Proteus mirabilis	31.25
Staphylococcus aureus	7.82
Klebsiella pneumoniae	7.82
Streptococcus species	31.25
Salmonella species	62.25

The Minimum Bactericidal Concentration (MBC) carried out in these studies determined the smallest concentration of the *Garcinia kola* aqueous stem extract that prevent growth of the bacteria isolates after incubating for 24hours. The stem extracts had MBC of 1.92mg/ml for *Klebsiella pneumonia*, 7.28mg/ml for *Escherichia coli* and *Staphylococcus aureus*, 125mg/ml for *Pseudomonas aeruginosa* 62mg/ml for *Salmonella species,* 15.68mg/ml for *Proteus mirabilis* and 31.25 mg/ml for *Streptococcus species* (Table 4).

Table 4: Minimum Bactericidal Concentration (MBC) of *Garcinia kola Stem* Extract on Bacteria Isolates.

Test Organisms	Concentration of Extracts in mg/ml (250-0.34)- MIC
Escherichia coli	7.28
Pseudomonas aeruginosa	125
Proteus mirabilis	15.63
Staphylococcus aureus	7.28
Klebsiella pneumoniae	1.92
Streptococcus species	31.25
Salmonella species	62.28

Discussion

The antimicrobial potentials of the stem extracts of *Garcinia kola* have been highlighted in this study. The research learned therefore and support the claims of folklore remedies and the potential of *Garcinia kola* as a multipurpose treatment for many ailments. It is worthy to note that the stem extract of *Garcinia kola* tested in this study were able to inhibit the growth of micro-organisms. The cold water, hot water, methanol extracts and ether extract of *Garcinia kola* stem tested on the selected bacteria isolates*; Escherichia coli*, *Pseudomonas aeruginosa, Proteus mirabilis, Streptococcus species, Staphylococcus aureus, Klebsiella pneumonia* and *Salmonella species* were found to inhibit the growth of the organisms with variable and considerable inhibition zone. In Nigerian homes today, herbal remedy from stem concoction is practiced as medicine by traditional healers Lemer, et al. [1991]. From the aqueous stem extract, phytochemical screening, showed that, it contains Tanin, Resin, Flavonoid, Cardiac glycoside, Alkaloid, Resin, Saponin and total absence of steroid in all the type of extraction which is indicative of the stem extract possessing bioactive constituent as its antibacterial and pharmacological source.

The aqueous stem extracts of 25mg/ml, 125mg/ml and 250mg/ml concentrations had antibacterial Activity Indices (AL) with Variable Inhibition Zones (IZ) to test bacteria isolates. The IZ of the various type of extraction for the different test bacteria isolates, had the maximum IZ range of 10-16mm for *Escherichia coli*, 13-16mm for *Staphylococcus aureus*, 6-14mm for *Proteus mirabilis,* and 7-20mm for *Klebsiella pneumonia*, with ether extract showing the highest capabilities to inhibit bacterial growth due to the present of significant secondary metabolites (phytochemicals) in the type of extraction, which are responsible for the medicinal properties of herbal extract and include, flavonoids, tannin, cardiac glycosides and resin. Mminimum antibiotic activity of the extract was seen in *Pseudomonas aeruginosa* of 7-11mm*, Streptococcus species* 6-12 and 9mm for *Salmonella species*. *Garcinia kola* compound is predominant by biflavanones and kolaflavanones which are major components of kolaviron [9], and aid folklore remedies for the treatment of various infections caused by pathogens. Flavoniod antimicrobial activities is compile with extracellular and soluble proteins in bacterial cell walls and act by inhibiting bacterial cell wall synthesis, resin (oleoresin) in the vessels of plants accounts for resistance to insect and fungus attack Drumond, Waigh, et al. (2000). Tanins are polymeric phenols constituent capable of precipitating gelatine from solution, a process known as astringency and are found in almost every plant bark Drumond, Waigh, et al. (2000). Broad spectrum antibiotics (Ciprofloxacin 5µg/ml) and narrow spectrum antibiotics (Tetracycline 30µg/ml) was used as positive control and gave IZ against all the test bacteria isolates ranging from 14-35mm and 9-23mm) respectively to the test bacteria isolates. The negative control (diluents used for extraction) i. e cold and hot water showed no IZ to bacterial isolates except 70% ethanol and ether which had 6-8mm inhibition zone to the bacteria isolates. The *Garcinia kola* stem extract Minimum Inhibitory Concentration (MIC) showed smallest concentration of the aqueous stem extract which showed minimum growth of the bacteria isolates when tested. The Bitter kola aqueous stem extracts had minimum inhibitory concentration of 7.28mg/ml for *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumonia*, *Streptococcus species* and *Proteus mirabilis* had MIC of 31.25mg/ml while *Pseudomonas aeruginosa* and *Salmonella species* had MIC of 62.25mg/ml respectively. The Minimum Bactericidal Concentration (MBC) in this study, showed that, 1.92mg/ml minimum concentration of the aqueous extract have bacteriocidal activity for *Klebsiella pneumonia*, 7.28mg/ml for *Escherichia coli* and *Proteus mirabilis* respectively, 15.68mg/ml for *Staphylococcus aureus*, 125mg/ml for *Pseudomonas aeruginosa,* 62.28mg/ml for *Salmonella species* and 62.28 mg/ml minimum concentration of the extract showed bactericidal activity for *Streptococcus species*.

Iwu [9] also reported that, the antibacterial effect of *Garcinia kola* antibacterial effect is believed to be largely due to Kalonones a secondary metabolite which predominates *Garcinia kola* compound seeds and stem. The Invitro studies of these extract have demonstrated that, considerably, it inhibits the growth of both pathogenic Gram positive and Gram-negative bacteria. These studies clarify it claims as broad-spectrum herbal remedy reported

by Reuter, et al. (1996). The antibiotic activities of the aqueous stem extract of *Garcinia kola* in this study, suggests that its stem extracts could attain therapeutic and bactericidal concentration in vivo. The inherent therapeutic property of *Garcinia kola* stem extract will be best attained when used alone. However, the combination of herbal extracts with antibiotics can result to partial or total synergism has reported by Didry, et al. (1992).

Conclusion

From the studies and results obtained, it can be concluded that the extracts of *Garcinia kola* stem extract inhibited the growth of bacteria. The minimum inhibitory concentration of the extract is suggestive of a good pharmacological source for palliative management of bacterial infection as herbal remedy. This in vitro study supports the claims of medicinal potential of traditional healers in the treatment of multipurpose ailments such as gastroenteritis, urinary tract infections, upper respiratory infections and wound infections as the aqueous stem extract contains secondary metabolites (phytochemicals) which make it effective against bacterial as contained in some convectional antibiotics prescribed for treatment in orthodox medicine.

Since the test isolates show various degree of susceptibility to the extracts, the laboratory should ensure continuous routine sensitivity test for each isolate and identification of these organisms to assert their antimicrobials sensitivity to herbal extract thereby saving cost of treatment of various infections. Bitter kola is not only limited to microbiology, but further work should also be carried out to determine the anticancer and antiviral properties. Other methods of extraction should be employed in extracting medicinal plants active components to determine the type of extraction having more bioactive components which can be a paramount research tool for pharmacognostics.

Conflict of Interest

The authors declare no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

Funding

This research did not receive any grant from funding agencies in the public, commercial, or not-for- profit sectors.

Acknowledgements

The authors would like to thank all the Laboratory and technical staffs of St Kenny Research Consult, Edo State for their excellent assistance and for providing medical writing support/ editorial support in accordance with Good Publication Practice (GPP3) guidelines.

References

1. Dalziel JM (1937) The Useful Plants of West Tropical Africa. Crown Agents for the Colonies, London.
2. Iwu MM (1993) Handbook of African medicinal Plants. Boca Raton, CRC Press Inc: pp. 223-224.
3. Adesina SK, Gbile ZO, Odukoya OA, Akinwusi DD, Illoh HC, et al. (1995) Survey of indigenous plants of West Africa with special emphasis on medicinal plants and issues associated with management. The United Nations Programme on Natural Resources in Africa 2nd (Edn) pp. 84-85.
4. Ayensu ES (1978) Medicinal Plants of West Africa. Refer Publ Inc Algonac Mich pp. 162.
5. Ajebesone PE, Aina JO (2004) Potential African Substitutes for Hops in Tropical Beer Brewing. J Food Tech in Africa 9(1): 13-16.
6. Nester EW, Roberts LE, Pearsall NN, Anderson DG, Nester MT, et al. (2004) Antimicrobial medicine: In Keven T. K., Ronald., E. N., Terrace, S., Jode, K. B. (eds). Microbiology A human perspective 2nd (Edn), Von Hoffman press Inc, University of Washington, New York. pp 447-460.
7. Okunji CO, Iwu MM (1991) Molluscidal activity of Garcinia kola biflavonones Fitoterapia 67: 74-76.
8. Terashima K, Kondo Y, Aqil M, Waziri M (1999) A study of biflavanones from the stem of Garcinia kola. Heterocycles 50: 238-290.
9. Iwu MM (1985) Antihepatotoxic constituents of Garcinia kola seeds and stem. Experientia 41(5): 699-700.
10. Trease GE, Evans WC (1983) Pharmacognosy. 14th (Edn), Publ Brown Publications.
11. Cheesbrough M (2000) summary of the clinical and laboratory features of microorganisms in: District laboratory practice in Tropical countries. Cheesbrough M (Edn) Part 2, Cambridge university press pp. 178-244.
12. Cowan ST, Steel KJ, (1965) Antibiotic sensitivity In: Cowan Steel's manual for identification. Cambridge university press, London, New York. pp. 24.
13. Ebana RU, Madunagu BE, Ekpe ED, Otung IN (1991) Microbiological exploitation of cardiac glycosides and alkaloids from Garcinia kola, Borreria ocymoides, Kola nitida and Citrus aurantifolia. J Appl Bacteriol 71(5): 398-401.
14. Iwu MM, Igboko OA, Okunji CO, Tempesta MS (1990) Antidiabetic and aldose reductase activities of biflavones of Garcinia kola. J Pharm Pharmacol 42(4): 290-292.
15. Okunji CO, Tantalia AW, Hicks RP, Iwu MM, Skanchy DJ, et al. (2002) Capillary electrophoresis determination of biflavonones from Garcinia kola in three traditional African medicinal formulations. Plant Med 68(5): 440-444.