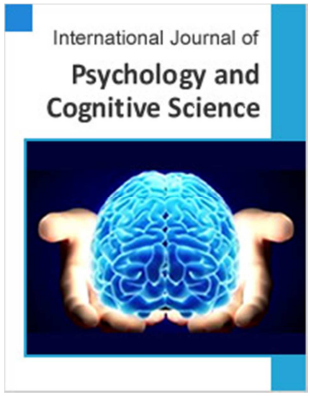
American Journal of Food, Nutrition and Health
2018; 3(1): 26-30
http://www.aascit.org/journal/ajfnh

AASCIT American Association for Science and Technology



Keywords
Garcinia kola,
Phytochemicals,
Antibacterial,
Gram Positive,
Gram Negative,
Amoxicillin,
Ampiclox

Received: January 12, 2018
Accepted: January 26, 2018
Published: February 12, 2018

Phytochemical and Antibacterial Activity of Methanol Extract of *Garcinia kola* Against Selected Bacteria Isolate

Saidu Talatu Bosso[1], Daniel Augustine Innalegwu[2]

[1]Department of Biological Sciences, Niger State Polytechnic, Zungeru, Nigeria
[2]Department of Biochemistry, Federal University of Technology, Minna, Nigeria

Email address
talatusaidu@yahoo.com (S. T. Bosso)

Citation
Saidu Talatu Bosso, Daniel Augustine Innalegwu. Phytochemical and Antibacterial Activity of Methanol Extract of *Garcinia kola* Against Selected Bacteria Isolate. *American Journal of Food, Nutrition and Health*. Vol. 3, No. 1, 2018, pp. 26-30.

Abstract
Plants have continued to be a good alternative in reducing the problem of cost and availability of drugs particularly to the developing world. Phytochemical and antibacterial activity of methanol extract of *Garcinia kola* against Gram positive *Streptococcus pneumoniae* ATCC 33400, *Streptococcus pyogenes* JCM 5674 and Gram negative *Pseudomonas aeruginosa* ATCC 10145, and *Klebsiella pneumoniae* BYK-9 was evaluated. Qualitative phytochemical screening revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids, anthraquinones, phlobatannins, steroids and terpenes while quantitative phytochemical analysis of the extract revealed the presence of phenol, flavonoid, tannin, saponin and alkaloid at a concentration of 524.011±18.85, 360.750±14.53, 224.625±1.94, 4.882±0.11 and 227.538±21.96 mg/100g respectively. Antibacterial activity of the extract at 20, 30 and 40 mg/mL revealed a spectrum of activity ranged between 10.33±0.67 to 21.33±0.33 mm in a concentration dependent manner which is significantly different ($p<0.05$) with 5 mg/mL of the standard antibiotics (Amoxicillin and Ampiclox). The MIC of the extract ranged between 0.625 to 5 mg/mL while the MBC ranged between 5 to 20 mg/mL on all the test bacteria isolates. Results of the study indicates that methanol extract of *Garcinia* kola contain useful phytochemicals that can be utilized in fighting infectious diseases.

1. Introduction

Plant derived medicines have continued to contribute immensely to human health and wellbeing from ancient times and have served as a good source for the synthesis of novel compounds used in orthodox medicines [1]. Based on the role they play in the development of new drugs they are categorized into two; they may serve as the analog in the synthesis of new drugs or as phytomedicine use for the treatment of disease or infections [1].

Over 80% of the world's population rely directly or indirectly on plant as their source of medication especially in the developing world [2]. Medicinal plants have been used as folklore remedies from ages to treat, manage or control man's ailment [3]. They are made up of varieties of chemical substances known as phytochemicals that possess important therapeutic properties used in the treatment of these ailments [4]. Also, the problem of multidrug resistance by microorganisms to commonly used antimicrobial agents have necessitated the search for newer and alternative compounds for the treatment of drug resistant infections and the high cost of conventional drugs,

particularly in the developing countries has led to the increased use of plants as an alternative for the treatment of infectious diseases [3]. Several researches on the chemotherapeutic potentials of plants have shown that plant can be a good source of antimicrobial compounds [5]. Since the beginning of human civilization, medicinal plants have been used by mankind for its therapeutic value. Nature has blessed mankind with plants as source of therapeutic agents for thousands of years and have continue to play a significant role as lead compounds in the modern pharmaceutical industries [3]. The motivating factor in the isolation of these compounds is based on their uses in traditional medicine [4]. According to the World Health Organization (WHO), a medicinal plant is any plant, which in one or more of its organ contain substances that can be used for therapeutic purposes or as precursors for drug synthesis [4]. Furthermore, WHO defines medicinal plants as herbal extracts produced by subjecting plants to physical or biological processes which may be produced for immediate consumption or as a basis for herbal products [3]. The continue rise in demand of plant-based medicines has mount pressure on some high-value medicinal plant populations in the world due to over-harvesting. A lot of these medicinal plant species are not ubiquitous, they have slow growth rates, low population densities, and are confined to some particular geographical locations [6] hence, vulnerable to extinction [7]. Also, due to poor documentation on the use of plant species for therapeutic and a bridge in the transfer of knowledge from the old generation to the new ones, man has continued to suffer from problems whose solution lies within reach [3]. Furthermore, the indigenous knowledge on the use of lesser-known medicinal plants is also rapidly declining due to civilization [3]. Finally, there is need to reinvestigated even the already tagged medicinal plants as some may have lost potency due to climate change.

The genus *Garcinia* belongs to the family *Clusiaceae* and is native to Asia, Australia, Africa and Polynesia. *Garcinia species* include evergreen trees and shrubs, dioecious and in several cases apomictic. Many species of *Garcinia* have fruit with edible arils, and most are eaten locally; some species' fruits are highly esteemed in one region, but unknown just a few hundred kilometers away. *Garcinia kola* is highly valued because of its medicinal use as the stem, root and bark serve as raw material for pharmaceutical properties. *Garcinia kola* is popular in south eastern Nigeria as it is extensively used in herbal medicine [8]. This study is therefore aimed at evaluating the phytochemical and antibacterial potentials of *Garcinia kola* (bitter kola) on some selected bacteria isolates.

2. Materials and Methods

2.1. Materials

2.1.1. Plants Materials

Fresh seeds of *Garcinia kola* (bitter kola) was obtained from Bosso Market in Niger State, Nigeria and was authenticated at the Biological Sciences Department of Federal University of Technology, Minna, Niger State.

2.1.2. Bacterial Strains

The bacteria strains used for the biological test were obtained from Federal Institute for Industrial Research Oshodi (FIIRO), Lagos, Nigeria. They include Gram positive *Streptococcus pneumoniae* ATCC 33400, *Streptococcus pyogenes* JCM 5674 and Gram negative *Pseudomonas aeruginosa* ATCC 10145, and *Klebsiella pneumoniae* BYK-9

2.2. Methods

2.2.1. Plant Processing and Extraction

Fresh seed of *Garcinia kola* (bitter kola) was washed, sliced into pieces and dried at the Drug Discovery Laboratory of Centre for Genetic Engineering and Biotechnology, Federal University of Technology, Minna, Niger State, Nigeria. The dried seeds were pulverized into fine powder using mortar and pestle. One hundred gram (100 g) of the plant powder was macerated exhaustively with 400 mL of absolute methanol at room temperature for 72 hours and filtered using whattman No 1 filter paper with pore size of 0.7µm to obtain a fine filtrate. The filtrate was then concentrated using RE- 6000 rotary evaporator and the solvent removed by the use of a thermostat controlled water bath at a temperature of 40°C to get the Methanol extract. The percentage yield of the extract calculated using the formula below:

$$\%\, Yield = \frac{Weight\ of\ extract}{Weight\ of\ dry\ sample}\ X\ 100$$

2.2.2. Qualitative Phytochemical Screening of Extracts

Preliminary qualitative phytochemical screening which involved performing simple chemical tests to detect the presence of secondary metabolites such as tannins, flavonoids, phenols, phenolic compounds, saponins, and glycosides, was carried out according to Trease and Evans and Sofowora [9], [10].

2.2.3. Quantitative Determination of Phytochemicals

Quantitative estimation of phytochemicals such as alkaloids and saponins was carried out according to [11], total phenolic content [12] and flavonoids using Aluminum Chloride colorimetric method [13].

2.2.4. Determination of Antibacterial Activity of the Extract

(i). Assay for Antibacterial Activity

Agar well diffusion method was used to evaluate the antibacterial activity of the Crude extracts [14]. Eighteen-hour culture of bacteria adjusted to 0.5 McFarland standard was used as inoculum on sterile Mueller Hinton agar. The plate was kept on flat bench for 30 minutes to solidify. Five

wells (4 mm) deep were made in the agar using a sterile 6 mm diameter cork borer. Then 0.5 mL of the reconstituted extract at a concentration of 20, 30 and 40 mg/mL was pipetted into the wells using micro pipette. Zero point five milliliter (0.5mL) each of 5mg/mL of Ampiclox and Amoxiline solution were used as positive controls and 0.5 mL of Di-Methyl Sulphoxide (DMSO) as a negative control. The plates were allowed to stand on a flat bench for 30 min to allow diffusion of the extract into the agar before incubation at 37°C for 24 h. Each test was carried out in triplicates and mean zone diameter of inhibition was recorded.

(ii). Determination of the Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the extract was determined using the double fold dilution. An aliquot of 1mL of the reconstituted extract with 50% DMSO at a concentration of 40 mg/mL was diluted serially to give concentrations of 20, 10, 5, 2.5, 1.25, 0.625 and 0.3125 mg/mL in eight test tubes. One milliliter (1 mL) of 18 h culture of bacteria previously adjusted to 0.5 McFarland standard (1.0×10^6cfu/mL) was added to each of the test tubes and the content was mixed thoroughly. The tubes were incubated at 37°C for 4 h. The 9^{th} test tube contained 1mL of 50% DMSO with no extract served as negative control. The 10^{th} and 11^{th} test tubes containing a solution of 5 mg/mL of Ampiclox and Amoxicillin served as positive control. The procedure was repeated for the test and the control. The test tube with the lowest concentration of the extract without visible turbidity of growth was taken as the MIC [15].

(iii). Determination of the Minimum Bactericidal Concentration (MBC)

From each of the test tubes without any visible growth, a loopfull of the broth was aseptically inoculated on a sterile Mueller Hinton agar. The inoculated plates were incubated for 24hr at a temperature of 37°C. After incubation, the MBC was determined as the lowest concentration with no visible growth on the plate [16].

2.2.5. Statistical Analysis

Data obtained in this study were analysed using the IBM Statistical Package for Social Science (SPSS) 20.0, 2011 version (SPSS Inc., Chicago, Illinois, USA) and Microsoft Excel Office 2013. Numerical data were presented as mean ± standard error of mean (SEM) of the triplicate.

3. Results

Table 1. *Qualitative phytochemical screening of methanol extract of Garcinia kola.*

Phytochemicals	Inferences
Alkaloids	+
Phenols	+
Tannins	+
Saponins	+
Cardiac glycosides	+
Phlobatannins	+
Anthraquinones	+
Steroids	+
Terpenes	+
Flavonoids	+

Key: + = Present

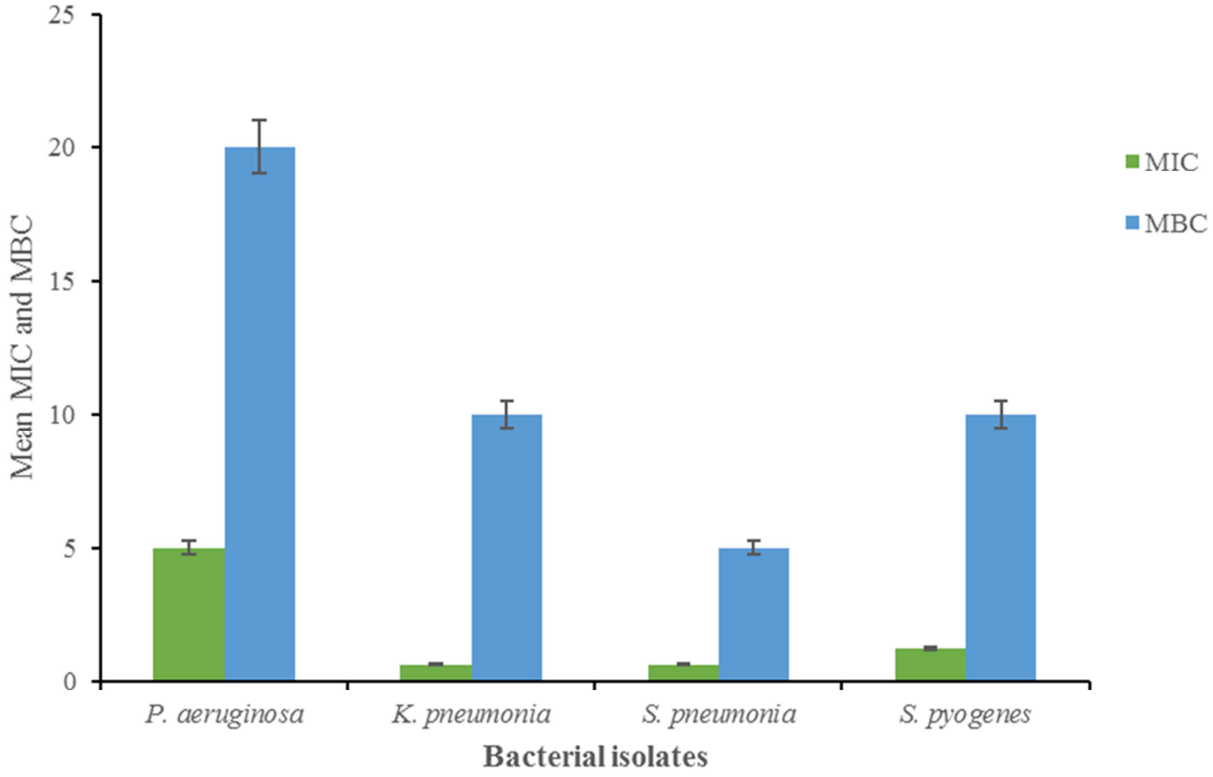


Figure 1. *MIC and MBC of methanol extract of Garcinia kola (Bitter kola) against bacterial isolates.*

Table 2. *Quantitative phytochemical analysis (mg/100g) of methanol extract of Garcinia kola.*

Total phenol	Total flavonoid	Alkaloid	Tannin	Saponin
524.011±18.85	360.750±14.53	227.538±21.96	224.625±1.94	4.882±0.11

Values are expressed in mean ± standard error of mean of duplicate determination.

Table 3. *Mean zones of inhibition of methanol extract of Garcinia kola (Bitter kola)*

Isolates	20mg/mL	30mg/mL	40mg/mL	Amoxicillin* 5mg/mL	Ampiclox* 5mg/mL
P. aeruginosa	10.33 ± 0.67^{a}	14.00 ± 0.58^{bc}	15.67 ± 0.33^{c}	13.67 ± 0.33^{b}	20.67 ± 0.33^{d}
K. pneumoniae	13.67 ± 0.67^{a}	16.33 ± 0.88^{b}	16.67 ± 0.67^{b}	19.00 ± 1.00^{c}	24.33 ± 0.33^{d}
S. pneumoniae	15.67 ± 1.20^{a}	19.00 ± 0.58^{c}	21.33 ± 0.33^{d}	$15.670.33^{b}$	24.33 ± 0.67^{e}
S. pyogenes	14.33 ± 0.88^{a}	19.67 ± 0.88^{b}	19.67 ± 0.67^{b}	26.00 ± 0.58^{c}	27.67 ± 0.33^{cd}

Values are expressed in mean ± standard error of mean, values with the same superscript on the same row have no significance difference ($p>0.05$), n=3
* Specification for Amoxicillin and Ampiclox are: ≤19 (resistance) and ≥20 (susceptible) (CLSI, 2012).

4. Discussion

The yield of an extract depends on the method of extraction, solvent used and the soluble constituent of the plant material in the solvent [18]. Methanol extract of *Garcinia* kola yielded 15.33%. Qualitative phytochemical screening of the extract revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids, anthraquinones, phlobatannins, steroids and terpenes (Table 1). Phytochemicals are used by plants either for defense against pathogens infestation or as waste exudate from the plant. Research have shown that these phytochemicals are useful as they are used to fight infections or diseases. The result of the qualitative phytochemical screening supports the findings of Oladunni *et al.,* (2017) [17], [8] and [20] who reported the presence of same phytochemicals in methanol extract of *Garcinia mannii* seeds, bark and root. Quantitative phytochemical analysis of the extract revealed the presence of phenol, flavonoid, tannin, saponin and alkaloid at a concentration of 524.011±18.85, 360.750±14.53, 224.625±1.94, 4.882±0.11 and 227.538±21.96 mg/100g respectively (Table 2). The amounts of phytochemicals present in plant varies from one organ to another as shown by [8] and [17] who compare the phytochemical constituent of seeds, stem and root of *Garcinia kola*. Variation in the amount of the phytochemicals may also be due to the type and quality of the extracting solvent as polar solvent have been reportedly used in extraction due to its high extracting power [19].

Antibacterial screening of the extract against *Streptococcus pneumoniae* ATCC 33400, *Streptococcus pyogenes* JCM 5674, *Pseudomonas aeruginosa* ATCC 10145, and *Klebsiella pneumoniae* BYK-9 at 20, 30 and 40 mg/mL revealed a spectrum of activity across all the test bacteria isolates. The zone of inhibition ranged between 10.33±0.67 to 21.33±0.33 mm in a concentration dependent manner which is significantly different ($p<0.05$) with 5 mg/mL of the standard antibiotics (Amoxicillin and Ampiclox). Similar spectrum of activity was also reported by [8] and [17]. MIC and MBC of the extract also indicate that the activity of the extract against *S. pneumonia* and *K. pneumoniae* with an MIC of 0.0625 mg/mL and an MBC of 5 and 10 mg/mL respectively. An MIC of 2.5 and 5 mg/mL was recorded for *S. pyogenes* and *P. aeruginosa* respectively while 10 and 20 mg/mL respectively was also recorded as the MBC (Figure 1). MIC and MBC of a plant extract is an indication of the activity of the extract. The lower the MIC and MCB value the higher the potency of the plant and therefore infer that methanol extract of *Garcinia* kola is active against these bacteria isolates and so can be a good alternative in fighting ailment cause by these pathogens. This research also justified the medicinal uses of *Garcinia kola* in treating infections caused by microorganisms are reported by [8], [17] and [19].

5. Conclusion

The antibacterial activity of methanol extract of *Garcinia kola* in this study explained their uses in traditional medicine. The presence and amount of phytochemicals such as phenol, flavonoid, tannin and saponins is an indication of the therapeutic potential of the plant as antibacterial agent.

References

[1] Evans, W. C. (2005). Trease and Evans pharmacognosy, 15th ed., Elsevier, pp. 20.

[2] WHO (2002). Traditional Medicine: Growing Need and potentials WHO policy perspectives on Medicines. World health Organization Geneva pp. 1-6.

[3] Akeem A., Enobong E. & Anthony A. (2014). *In Vitro* Evaluation of the Antimicrobial Potentials of the Leaves Extracts of *Distemonanthus benthamianus* (baill) and Its Interaction with Amoxycillin. *Current Trends in Technology and Science,* 3: 3.

[4] Doughari, J. H. (2009). Phytochemicals: Extraction Methods, Basic structures and Mode of Actions as Potential Chemotherapeutic Agents. pp. 1-33.

[5] Ngulefack, E. M. P., Ngu, K. P., Atchade, A., Dimo, T., Tsabang, N. & Mbafor, J. T. (2005). Phytochemical composition and *in-vitro* effects of the ethylacetate bark extract of *Distemonanthus benthamianus* Bailon (Caesalpiniacea) on *staphylococcus aureus* and *streptococcus agalactiae*. *Cameroon Journal of Experimental Biology,* (1): 50-53.

[6] Nautiyal, S., Rao, K. S., Maikhuri, R. K., Negi, K. S. & Kala, C. P. (2002). Status of medicinal plants on way to vashuki jal in Mandakini valley, Garhwai Uttaranchal Journal of Non-Timber Forest Products. 91: 124-131.

[7] Jablonski D. (2004). Extinction: Past and present. *Nature*, 427: 589. doi: 10.10381427589a [Pubmed].

[8] Ukaoma, A. A., Ukaoma, V. O., Okechukwu, R. I. & Iwuagwu M. (2013). Phytochemical screening and antibacterial properties of *Garcinia kola. The Journal of Phytopharmacology*, 2 (3): 34-38.

[9] Trease, G. E. & Evans, W. C. (2002). Pytochemistry: Introduction in General Methods. *Pharmacognosy*, 11th ed. Bailliere Tindll., London, pp. 227-247.

[10] Sofowora, E. A. (1982). Medicinal plants and Traditional medicine in Africa," John Wiley and Sons Ltd, New York, pp. 256-257.

[11] Oloyed, O. I. (2005). Chemical profile of unripe pulp of *Carica pagaya. Pakistan Journal of Nutrition*, 4: 379-381.

[12] Singleton, V. L., Orthofer, R. & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology,* 299: 152-178.

[13] Chang, C., Yang, M., Wen H. & Chern, J. (2002). Estimation of total flavonoid content in *Propolis* by two complementary colorimetric methods. *Journal of Food Drug Analysis*, 10: 178-182.

[14] Akinyemi, K. O., Oluwa, O. K. & Omomigbehin, E. O. (2006). Antimicrobial Activity of Crude Extracts Of Three Medicinal Plants Used In South-West Nigerian Folk Medicine On Some Food Borne Bacterial Pathogens. *African Journal of Traditional, Complementary and Alternative Medicines.* 3 (4): 13-22.

[15] Cheesebrough, M. (2006). District Laboratory Practices in Tropical *Countries*, Cambridge University Press, Edinburgh, United Kingdom, pp. 382-407.

[16] De, N., Ifeoma, E. (2002). Antimicrobial effects of components of the bark extracts of neem (*Azadirachta indica* A Juss). *Journal of Technical development,* 8: 23-28.

[17] Oladunni, A., Garba, S. A., Musa, G., & Audu J. A. (2017). Phytochemical Screening and Antibacterial Activity of Extracts of *Garcinia mannii* and *Terminalia avicennoides* on some oral bacterial pathogenes. *AASCIT Journal of Health,* 4 (2): 8-16.

[18] Sarker, D. S., Latif, Z., & Gray, I. A. (2006). Natural Products Isolation. Second Edition. p. 4-70.

[19] Mboto, C. I., Eja, M. E., Adegoke, A. A., Iwatt, G. D. Asikong, B. E., Takon, I., Udo, S. M., & Akeh, M. (2009). Phytochemical properties and antimicrobial activities of combined effect of extracts of the leaves of *Garcinia kola, Vernonia amygdalina* and honey on some medically important microorganisms. *African Journal of Microbiology Research,* 3 (9): 557-559.