PRINT ISSN 1119-8362
Electronic ISSN 2659-1502

Full-text Available Online at
https://www.ajol.info/index.php/jasem
http://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 27 (7) 1379-1385 July 2023

Antibacterial and Bioactive Properties of Methanol and Ethanol Extracts of the *Materia medica Garcinia kola* (heckel)

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ABSTRACT: This paper investigated the antibacterial properties of methanol and ethanol extracts of bark, leaves, pod, root and seed of Garcinia kola using appropriate standard techniques. Data obtained show that the methanol extract from samples of *Garcinia kola* contain higher percentage concentration of phytochemicals than ethanol. Saponins (4.20±0.04) occurred highest in the bark, alkaloids (1.54±0.81) in the seed, tannins (1.96±0.17) in the pod, flavonoids (0.92±0.14) in the leaves, while the root contain highest percentage of phenol (0.44±0.11). It was observed that the bark extract of *Garcinia kola* was more effective in inhibiting the growth of *Pseudomonas auraginosa, Esherichia coli* and *Staphylococcus aureus* when compared with the extract from the leaves, pod, root and seed. The inhibition of the test extract was higher in extract from the bark, followed by the seed, root, pod and the least zone of inhibition was found in the leaves extracts. The variation in the antibacterial activities is presumed to be due to different active compounds present in the extract. Concentrations of essential organic compounds occurred most in the seed with carbohydrates been dominant, while lipids remains the least concentrated. The appreciable concentrations of phytocompounds detected in the samples of the studied bioresource, coupled with the medicinal potentials are an indication of the plant effectiveness in nutraceutical applications.

DOI: https://dx.doi.org/10.4314/jasem.v27i7.7

Cite this paper as: OJATULA, A. O; OLAFUSI, G. T; ADETUTU, H. B. (2023). Antibacterial and Bioactive Properties of Methanol and Ethanol Extracts of the *Materia medica Garcinia kola* (heckel). *J. Appl. Sci. Environ. Manage.* 27 (7) 1379-1385

Dates: Received: 30 May 2023; Revised: 22 June 2023; Accepted: 02 July 2023 Published: 30 July 2023

Keywords: *Garcinia kola*; *Pseudomonas auraginosa; Esherichia coli;* nutraceutical

The medicinal values of plants lie in the chemical substances presents in the parts of the plant such as seed, leaves bark and root (Ojatula, 2019). These substances produce definite physiological action in the human body, thereby, bringing about holistic healing of human, animal inclusive (Ojatula, 2022; Ojatula and Ikuesan, 2022; Ojatula and Afolabi, 2022). Plants have immense impact on the nutrition and health of human exhibiting curative tendency for ailments. Plants contain a broad range of biochemically active compounds which are the primary sources of nutrition and various medicines. Wild plants including those that are under-utilized are sources of nutraceuticals providing vitamins, trace elements and minerals, thereby improving health and nutrition (Leonti, 2012; Ojatula, 2021). Several traditionally known medicinal plants contain several different pharmacologically active compounds that may act individually or synergistically to improve health and function as food for growth and body maintenance (Afolayan and Jimoh, 2009; Ubwa *et al*., 2014; Verma and Kaushal, 2014; Ojatula *et al*., 2019). Researchers' attention focuses on investigating each plant with a new approach regarding their probable use for food and medicine. Verma and Kaushal, (2014) reported that essential organic compounds (carbohydrate, proteins, vitamins, steroids and lipids) of plant origin are primary metabolites for source of food and health

enhancement in human nutritional science, animal inclusive, while in literature, others stated that secondary metabolites, such as phenols, flavonoids, tannins, alkaloids, terpenoids, lignin, quinones, coumarins and amines function optimally as antioxidants, antimicrobial and antipyretic (Zheng and Wang, 2001; Ojatula, 2019; Ojatula *et al*., 2023). Similarly, many plants (wild or domesticated) provide energy, vitamins and minerals that maintain the physiological equilibrium of the body (Ojatula and Ikuesan, 2022). Ebert (2014) however, reported that the nutritional potential of uncultivated plant species is superior relative to the cultivated variety. Several ethno- pharmacological researches on wild edibles as nutraceuticals used for the cure of jaundice, diabetes, wounds, cancer e. t. c have also received great attention (Mir, 2014). Foods of plant origin are abundant resources of bioactive compounds which possess a great variety of biological activities including antioxidant potential. Fruits and vegetables as food result in decline possibility of chronic and neurodegenerative diseases, mostly due to the occurrence of antioxidants (phenolic compounds and tocopherols) by interruption or prevention of oxidative reactions (Di Matteo and Esposito, 2003). The occurrence of infectious diseases is increasing globally and the use of antimicrobials for cure is trending. Plant antimicrobials are naturally occurring secondary metabolites such as tannins, flavonoids, quinones, coumarins, terpenoids, alkaloids and polypeptides which exert innocuous antimicrobial activity compare to man-made or synthetic drugs (Savoia, 2012). From native accounts, the use of natural substances, particularly of plant origin, for preventive and curative potentialities is common and used for holistic healing. These plants possess phyto-compounds at varying qualitative and quantitative concentrations which account for their use as plant possessing nutraceutical values. Despite modern development in the treatment of diseases, the use of herbal remedies have been continuous and universal (Erhabor *et al*., 2015). Modern medicines have always depended on herbal extracts from plants as fundamental source of therapeutic ingredients (Ojatula *et al*., 2020). Some naturally occurring substances in plants play significant role in plant disease resistance and thus most bacteria are sensitive to extract from these plants (Ojatula and Ikuesan, 2022). Plants are pools of potential antimicrobial compounds for pharmaceutical need. The array of active compounds derived from them have impressive pharmaceutical properties such as analgesics, aesthetic, antibacterial, antifungal, antibiotics, anti–parasitic, anti-inflammatory, oral contraceptive, hormones, and ulcer therapeutic laxative (Ojatula *et al*., 2023). Among the many medicinal plants possessing such healing potentials as claimed by the locals is *Garcinia kola*; and a great variety of phytochemical compounds such as alkaloids, tannins, flavonoids, saponins, and phenolic compounds are found accumulating in the *Materia medica* of plant origin (Okwu, 2003). *Garcina kola* is a species of flowering plant belonging to the *Clusiaceae* or *Guttiferae* family. It is often called bitter kola (English), Orogbo (Yoruba), Aka ilu (Igbo) and Namijin goro (Hausa). It is an indigenous medicinal plant found in rain forest of central and western Africa, especially Benin, Cameroon, Democratic Republic of Congo, Cote d'Ivoire, Gabon, Ghana, Liberia, Nigeria, Senegal and Sierra Leone. It is a medium sized evergreen tree, with height of about 15-17 m and a fairly narrow crown. The leaves are simple, 6-14 cm long and 2-6 cm across, shiny on both surfaces and spotted with resin glands. The small flowers are covered with short, red hairs (Iwu, 1993). *Garcina kola* fruit is a drupe of 5-10 cm in diameter and weight between 30 to 50 g. The fruit changes colour during maturation from green to orange, and each fruit contains 1-4 smooth elliptically shaped seeds (Juliana *et al*., 2006). *G. kola* is an important component of traditional herbal medicine and has been referred to as "wonder plant" because every part of the plant from the root to its seeds has great medicinal importance (Esiegwu *et al*., 2014). When eaten, the seed has a bitter astringent taste. The use of *G. kola* seed in folk medicine and several herbal formulations have been reported together with its potential therapeutic benefits due mainly to the presence of flavonoids and other bioactive compounds (Akintonwa and Essien, 1990; Tona *et al*., 1999). *G. kola* is used in folklore remedies for medicinal treatment as purgative, antiparasitic, antimicrobial, anti-inflammatory, gastroenteritis, rheumatism, asthma, menstrual cramps, bronchitis, throat infections, headache, coughs and liver disorders (Iwu, 1985). Also the plant has been used as an aphrodisiac (Erhabor *et al*., 2015), antidiabetic, antioxidant and for the chemoprevention of aflatoxin B1 and antihepatotoxic activities (Braide, 1991; Farombi *et al*., 2005). Although reports exist on various pharmacological and nutritional activities of *G. kola*, but literature reveals not enough information on its *materia medica*. This study therefore investigates the antibacterial properties of methanol and ethanol extracts of bark, leaves, pod, root and seed of *Garcinia kola*

MATERIALS AND METHODS

The current study was experimental with the aid of wire loop on the isolates of the bacteria which were aseptically streaked in a nutrient agar media, and the plant extracts were tested at various concentrations justifying human circumstances.

Sample Procurement and Preparation: The sample used in the research work was obtained from Eureka Herbal Garden, IgboEgunrin, Ilaje Local Government Area, Ondo State, Nigeria, while the laboratory used for the practical was the Central Laboratory Service Unit of Federal University of Technology (FUTA), Akure, Ondo State. The leaves, seeds, pods, bark and root of *Garcinia kola* were used for the analyses. The leaves and seeds of *Garcinia kola* were harvested and placed into a neatly washed and dried tray. The leaves, seeds and the pods were cut into bits for fast drying. The leaves were air dried; while seeds and pod were sun dried and both crushed to coarse powder using a neatly washed local mortar and pestle. The bark of the *Garcinia kola* was thoroughly washed to remove the sand, after which it was cut into bits to aid fast drying. The same procedure was also carried out for the root. The powdered form of the leaves, seed, pod, root and bark of *Garcinia kola* were placed in different containers and were properly labelled for further experimental usage. Clinical pure isolates of *Staphlococcus aureus*, *Echerichia coli*, and *Pseudomonas auroginosa* were subculture to obtain pure isolates and identified on the basis of the colonial reactions and biochemical characteristics.

Extraction: Absolute ethanol 90% and methanol were used as solvent, for extraction of active compounds in the plant materials. 15 g of each processed plant materials of *Garcinia kola* was soaked separately in 100 ml of 90% absolute ethanol in 250 ml flask. The same was done with methanol. This was shaken vigorously and allowed to stand for 72 hours to effect proper extraction of active ingredient. The suspension was filtered with Whitman's No. 1 filter paper to obtain the supernatant while the debris was discarded. The extract after distillation was analysed according to standard laboratory analytical procedures outlined, described and published (Harbone, 1973; Ojatula, 2021; Ojatula, 2022) for the assay of phytochemicals.

Determination of essential organic compounds (biomolecules) of *Garcinia kola* was done in accordance with the standard laboratory analytical methods described by Oluduro (2012); and Ojatula (2019). Representative subsamples were dried in a forced drought oven at 105-110 ^{o}C to a constant weight. Protein and lipids were analysed by triplicate according to the aforementioned methods. The total carbohydrates were determined by the difference method [100 – (protein + lipids in percentage)].

Standard filter paper was used to prepare 2 mm paper disc with the aid of perforating machine. These discs were thereafter sterilized by autoclaving at 121 ^{O}C for 15 minutes and later interpregnated with the plant extracts under aseptic conditions. The antibacterial activity of the different extracts was determined against test organisms using agar diffusion method. 0.2 ml of a 24 hours subculture of each test organisms was uniformly spread over the surface of a sterile nutrient agar and allowed to dry. The bacteria isolated were first sub-cultured in a nutrient agar. Serial dilution of the plant extracts was made. Number 4 filter papers were perforated with the aid of paper perforators. These filter papers now in the form of disc perforated out were impregnated into the serial dilutions of the plant extracts with various concentrations of 1.0, 0.8, 0.6, 0.4, 0.2 ug/dl. The plant extracts were tested at various concentrations. With the aid of wire loop, the isolates of the bacteria were aseptically streaked in a nutrient agar media. The prepared different concentrations of the plant extracts with the aid of a forceps were placed on the surface of the streaked nutrient agar media. The agar plates were randomly placed in the oven incubated at 37 ^{O}C for 24 hours. The impregnated nutrient agar media were placed in an incubator at 37 ^{O}C for 24 hours. The experiments were done in triplicates. The zones of inhibition were measured in mm diameter and recorded.

RESULTS AND DISCUSSION

The minimal inhibitory concentration of the isolates are shown in Tables 1. While the zones of inhibition of the isolates are shown in Table 2. Results show that *Pseudomonas auroginosa* was more sensitive when compared with other bacteria isolates tested; and the degrees of sensitivity were found occurring on the bark extract than the extracts of leaves, seeds, pod and root.

Table 1. Minimum Inhibitory Concentration (MIC: mg/ml) of the extracts of *Garcinia kola*

Bacteria	Extract	Leaves	Seeds	Pod	Bark	Root
Staphylococcus	Methanol	0.05	0.06	0.06	0.07	0.07
aureus	Ethanol	0.01	0.02	0.03	0.03	0.01
Esherichia coli	Methanol	0.06	0.08	0.07	0.11	0.08
	Ethanol	0.05	0.05	0.04	0.08	0.03
Pseudomonas	Methanol	0.06	0.11	0.09	0.90	0.09
auraginosa	Ethanol	0.04	0.09	0.06	0.50	0.05

The antibacterial activity of the extracts can be attributed to the synergistic action of some bio-reactive substances such as the alkaloids, tannins, saponins, flavonoids among others in the extracts (Okwu, 2003; Ojatula and Ikuesan, 2022). Many higher plants are known to possess antibacterial agents and indeed extracts of plants from different parts of the world have been known to produce antimicrobial properties as observed in this work; and this agrees with the findings of Okwu (2003) while working on the medical and nutritional potentials of *Garcinia kola*. Table 2 shows the zones of inhibition of the extracts on the test organisms. Extracts from *Garcinia kola* bark generally had higher zones of inhibition on all the tested isolates, although it was more effective on *Pseudomonas auraginosa* (42.5 mm) than other isolates. Also (table 2) shows the zones of inhibition of the test organism by extracts of the leaves, seeds, pod, bark, and the root of *Garcinia kola*. It was observed that the inhibition of the tested extracts in millimetre was higher in the bark, followed by the seed and the least zone of inhibition was found in the root extract. This shows that *G. kola* can be extensively used in treatment of bacteria disease/infection, particularly with the bark extract (Agu and Ogu, 1995).

Table 2. Zones of Inhibition (mm) of the extracts of *Garcinia kola*

Bacteria	Extract	Leaves	Seeds	Pod	Bark	Root
Staphylococcus	Methanol	16.05	21.5	21.3	21.50	17.5
aureus	Ethanol	15.01	19.5	18.03	20.5	16.0
Esherichia coli	Methanol	15.6	17.5	17.4	25.0	20.50
	Ethanol	12.1	15.0	16.0	23.5	18.0
Pseudomonas	Methanol	23.25	30.25	30.09	42.50	35.0
auraginosa	Ethanol	18.10	29.0	25.06	40.0	34.0

It was also shown that the methanol and ethanol extracts of *Garcinia kola* inhibited the growth of gram positive and the gram negative organism. Although extract produced with methanol exhibited more inhibitory effect when compared with ethanol, this could be because of high active compounds present in the extract. The result in this work shows that there is variation in the degree of antibacterial activities of the extracts. The variation in the antibacterial activities is presumed to be due to difference in the quantity of compounds present in those plant extracts. The results obtained on bacteria test isolates indicated that the lethal effect was greater on *P. auroginosa* (45.6 mm). Attempt has been made to show that the crude nature of this extracts as well as the age long problem of dosage is obstacle in the general acceptance of this plant extract. The presence of impurity, which may negatively modify the effect of this extracts may ultimately lead to loss of therapeutic value. Although results have revealed the potentials of this medicinal plant, *Garcinia kola*, as having antibacterial effect, with the possibility of complementing the existing literature, and giving future hope on its bioactivity and efficacy in combating the menace, thereby leading to greater acceptability of the herbal medicine in health sustainability by majority of the populace. The Phytochemical compositions of *Garcinia kola* extracts of the leaves, seed, pod, bark, and root are shown in Table 3, while the quantitative phytochemical components in percentage are shown in Table 4. Result showed that the plant material samples of *Garcinia kola* contains phytochemical of interest, such as alkaloids, tannins, flavonoids and saponins in different concentrations. These identified bioactive principles had been implicated in the bioactivity and efficacy of extracts of natural sources, plants in particular (Ojatula, 2019; Ojatula, 2021). The bark extracts containing higher quantity of saponins, flavonoids, tannin, alkaloid and phenol. The table also shows that the active compound tannins, saponins and alkaloid were more abundant in the extract of methanol than the ethanol. The presence of alkaloids in the sample of *Garcinia kola* agrees with the findings by Oyeleke *et al.* (2008), that the antibacterial activity of this plant may be attributed to the presence of alkaloids. And that pure isolated alkaloids and their synthetic compounds have been used in medicine as bactericidal agents (Oyeleke *et al*., 2008). Saponins derived from fruits and vegetables are important dietary supplements with antimicrobial properties that protect plants from microbial pathogens (Sczkowski *et al*., 1998). Phenols found in natural sources of plant origin are used in the treatment and management of cancer, ulcers, and malaria. They also have medicinal properties like anti-carcinogenic, antimalarial, anti-ulcer, antimicrobial, and diuretic activity (Dudareva *et al*., 2004). As a result of the presence of these bioactive compounds, the medicinal plant possessing them, *Garcinia kola*, could be used in no small measure in ethnomedicine to treat a variety of ailments. Table 4 shows the percentage (%) active compound in the extracts of ethanol and methanol. The result showed that the active compounds extracted was higher in the extract of bark than those of the leaves, seed, pod and root. The bark produced highest active compound of saponins (4.2%) than those of the leaves, seed, root and pod (3.10, 1.76, 1.54 and 1.34 respectively).

Table 3. Phytochemical composition of *Garcinia kola*

Sample	Extracts	Tannins	Flavonoids	Saponins	Alkaloids	Phenol
Leaves	Methanol	+	++	++	++	++
	Ethanol	+	++	++	++	++
Seeds	Methanol	+++	+	++	+++	++
	Ethanol	+	+	++	+	+
Pod	Methanol	+++	+	++	+	++
	Ethanol	+	+	++	+	+
Bark	Methanol	+++	++	+++	++	+
	Ethanol	++	++	++	++	+
Root	Methanol	+++	+	++	+	++
	Ethanol	++	+	+	+	+

Key: *+ = presence of photochemical, ++ = strong presence of photochemical*
+++ = abundant presence of photochemical

Table 4. Quantitative photochemical components of *Garcinia kola* in %

Sample	Solvents	Tannins	Flavonoids	Saponins	Alkaloids	Phenol
Leaves	Methanol	1.02±0.03	0.92±0.04	3.10±0.04	1.34±0.81	0.13±0.010
	Ethanol	0.03±0.02	0.44±0.01	2.30±0.03	1.03±0.72	0.16±0.003
Seeds	Methanol	0.98±0.02	0.34±0.04	1.76±0.03	1.57±0.82	0.35±0.009
	Ethanol	0.44±0.01	0.22±0.02	1.32±0.02	0.03±0.02	0.14±0.007
Pod	Methanol	1.96±0.17	0.18±0.02	1.34±0.4	0.78±0.02	0.42±0.01
	Ethanol	0.46±0.07	0.14±0.01	1.13±0.3	0.17±0.01	0.16±0.07
Bark	Methanol	1.77±0.03	0.47±0.03	4.20±0.04	1.24±0.81	0.35±0.010
	Ethanol	0.70±0.01	0.47±0.01	2.70±0.03	1.03±0.72	0.13±0.007
Root	Methanol	0.96±0.03	0.78±0.02	1.54±0.4	0.077±0.02	0.44±0.11
	Ethanol	0.03±0.02	0.17±0.01	1.13±0.3	0.025±0.01	0.16±0.07

Results showed that methanol and ethanol used as solvents were effective in extracting the active compounds with methanol being more effective in extracting more active compounds as shown in table 4 particularly saponins (4.20±0.04) and alkaloid (1.54±0.81). This could be because of high volatile property of methanol. Results of phytochemical analysis revealed the predominance of active components assayed in both ethanol and methanol extracts of *Garcinia kola*.

Table 5. Essential organic compounds of *Garcinia kola* in %

Sample	Carbohydrates	Lipids	Proteins
Leaves	4.12±0.03	0.02±0.01	0.19±0.13
Seeds	6.25±1.57	0.03±0.01	1.99±0.13
Pod	6.05±1.51	0.03±0.01	1.59±0.03
Bark	4.82±0.09	0.02±0.01	0.39±0.03
Root	5.25±1.57	0.01±0.01	0.29±0.10

Furtherance to the results obtained in this study Table 5, it can be deduced that *Garcinia kola* contains appreciable nutritional compounds making it to be a good source of food supplement. Appreciable level of crude nutrients in natural sources of plant origin is acceptable as it could prevent the occurrence of diseases thereby promoting good health and well-being for the living (Ojatula and Enoyoze, 2023; Ojatula and Ezenwa, 2023). Carbohydrate deficiency causes depletion of body tissues. Sufficiency of carbohydrate is however, necessary for optimum functioning of the brain, heart, nervous, digestive and immune system (Baker, 1996). The percentage concentration of biomolecules recorded in this study compete favourably and relatively valuable with that obtained in similar researches published by Anthonia (2002); Oluduro (2012) and Ojatula (2019). The variations in nutritional makeup of the samples of *Garcinia kola* analyzed in this study and that of other similar researches on *G. kola* could be attributed to the differences in genetic makeup of the plant material, and varying climatic and edaphic factors. Therefore, the presence of specific types of molecules (the biomolecules) and of certain metabolic pathways are invariant features among the wide diversity of life forms; thus these biomolecules coupled with metabolic pathways in organisms are referenced as bio-compounds of material unity of the living beings which are essential to one or more typically biological processes, such as cell division, morphogenesis, or development for essential physiological and metabolic processes and functioning (Ojatula, 2019).

Conclusions: Our study suggests that the observed inhibitory effect of extracts of *G. kola* explained their utilization in traditional medicine; of which, the zones of inhibition as observed in the results of this study, implicated *G. kola* possessing potent antiseptic or antibacterial properties; and that findings from this work support the use of extracts from *G. kola* in treating wound as it prevents the wound from being septic. Also, the appreciable concentrations of phytocompounds detected in the bio-resource, depicts an indication of the plant in nutraceutical applications.

Acknowledgement: The authors would like to express their gratitude to the staff of the Central Research Laboratory, Federal University of Technology

(FUTA), Akure, for their tremendous cooperation and assistance. They are grateful to management for providing infrastructure, research, and laboratory facilities for conducting experimental studies in accordance with the National Research Council (NRC). We also express our heartfelt gratitude to those not mention but definitely not forgotten.

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