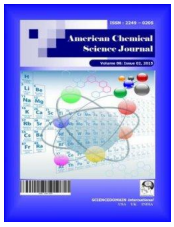

American Chemical Science Journal
13(4): 1-7, 2016, Article no.ACSJ.24019
ISSN: 2249-0205

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Chemical Constituents and Antimicrobial Activity of the Leaf Essential Oil of *Garcinia kola* Heckel (Clusiaceae) from Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author SEO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author PO performed the antimicrobial and antimycobacterial study. Authors HOE and OFK managed the analyses of the study. Author CIB managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ACSJ/2016/24019
Editor(s):
(1) Mohammad Luqman, Department of Basic Science, College of Applied Sciences, A'Sharqiyah University, Oman.
(2) Anonymous.
(3) Say Leong Ong, Civil and Environmental Engineering Department and NUS Environmental Research Institute, National University of Singapore (NUS), Singapore.
Reviewers:
(1) Marco Matteo Ciccone, University of Bari, Italy.
(2) Francisco Jose Torres De Aquino, Universidade Federal De Uberlandia, Brazil.
(3) James Adams, University of Southern California, USA.
Complete Peer review History: http://sciencedomain.org/review-history/14009

Original Research Article

Received 31st December 2015
Accepted 3rd March 2016
Published 5th April 2016

ABSTRACT

All parts of *Garcinia kola* are used in traditional medicine for various ailments including inflammation and infections. The leaf essential oil (EO) of *Garcinia kola* was isolated by hydrodistillation using Clavenger type apparatus and subjected to GC-MS analyses. A total of twenty-seven compounds were identified representing 88.27% of the oil. The main components of

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the essential oil were citronellic acid (48.3%), 5,9-undecadien-2-one (5.12%), α-ionone (4.38%), 3-buten-2-one (3.25%), 2-pentadecanone (2.59%), squalene (2.27%), nonacosane (2.18%), octanal (1.9%), geraniol (1.52%), mesitylene (1.17%) and α-farnesene (1.02%). The oil contained many compounds that were active against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli*, *Candida albicans* and *Mycobacterium bovis* with minimum inhibitory concentrations ranging from >400 to 50 µg/ml and could be exploited for the development of plant-based drugs.

Keywords: Garcinia kola; Clusiaceae; leaves; essential oil; antimicrobial; citronellic acid.

1. INTRODUCTION

Garcinia kola, a dicotyledonous plant belonging to the Clusiaceae/Guttiferae family is commonly found in the subtropical and tropical forests of some countries of West and Central Africa [1]. The genus Garcinia consists of more than 300 species. The genus is a native of Asia and Africa and consists of evergreen polygamous trees, shrubs, and herbs. About 35 species are reported to exist in India, many of which are endemic and economically important with immense medicinal properties [2]. In Eastern part of West Africa there are over fifty species of kola. In Nigeria, there are about twenty-three species, out of which five are edible [3]. *Garcinia kola* is a medium-sized evergreen tree, about 15-17 m tall and with a fairly narrow crown (Fig. 1), the seeds are shown in Fig. 2. 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 [4]. *Garcinia kola,* locally known as "Namijin goro" among the Hausas of Nigeria and commonly called bitter kola belongs to a class of plants described as masticatory. A wide range of medicinal uses of *G. kola* as reported in the literature include its use as antiparasitic, antimicrobial, antiviral, anti-inflammatory, purgative, antidote to the effects of *Strophanthus gratus*, remedy for guinea-worm infection and for the treatment of gastroenteritis, rheumatism, asthma, menstrual cramps, bronchitis, throat infections; cure for head or chest colds, cough, and liver disorders. The plant is also used as antidiabetic, antioxidant, and for the chemoprevention of aflatoxin B1 and antihepatotoxic activities [5]. The seed and leaf of the plant are used in folk medicine to treat gram positive and negative bacteria, Ebola virus infections, flu, dysentery and diarrhea [6]. The leaves are also used to treat stomach problems and typhoid [7]. The leaf extract of *G. kola* produced antioxidant effect and hence can induce protective response against the destructive effects of free radicals on both brain and liver [8]. It has also occupied a place in an ancient Indian Ayurveda as a purgative and as an aid that activates digestion. [9] The phytochemicals obtained from *G. kola* include biflavonoids, xanthones, kolanone, ameakoflavone, coumarine and prenylated benzophenones. [5] Two new chromanols, garcinnoic acid, garcinial, together with 8-tocotrienol were reported. *G. kola* also contains tannins, cardiac glycosides, saponins, alkaloids, hydroymethyl anthraquinones, polyphenols, glucosides and reducing compounds [10]. The aim of this study is to evaluate the chemical constituents and antimicrobial activity of the leaf essential oil of *Garcinia kola* grown in Nigeria.

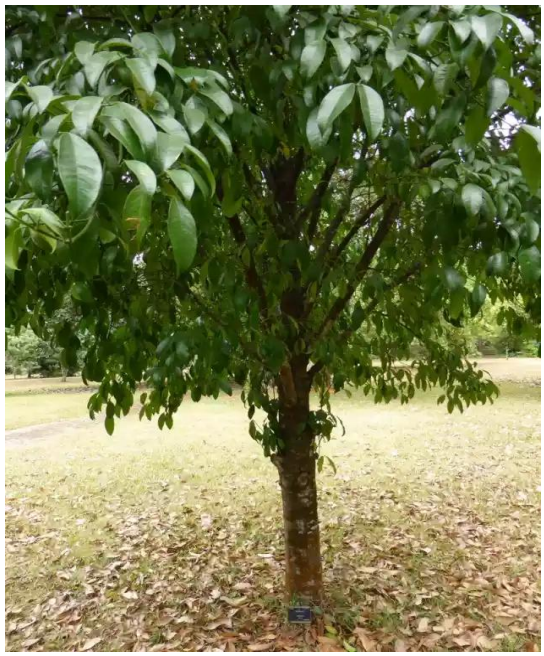

Fig. 1. The whole tree of *Garcinia kola*

Fig. 2. Seeds of *Garcinia kola*

2. MATERIALS AND METHODS

2.1 Plants Materials

The plant material was collected from adult trees in June 2015 from Oforisi village, Okigwe, Imo State Nigeria. Dr. Grace Ugbabe of the National Institute of Pharmaceutical Research and Development, Idu, Abuja, Nigeria, identified the plant material and a voucher specimen has been deposited at the herbarium under voucher number NIPRD/H/6656.

2.2 Essential Oil Isolation

The fresh leaf sample (500 g) was chopped into small pieces and hydrodistilled using a Clavenger type apparatus for 4 h and yielded a golden yellow essential oil (0.5 ml). The golden yellow essential oil obtained was dried over anhydrous sodium sulphate and stored at 4ºC in sealed vials until analysis.

2.3 Gas Chromatography- Mass Spectrometry Analysis

The oil was analyzed by GC-MS using Shimadzu QP-2010 GC with QP-2010 mass selective detector [MSD, operated in the EI mode (electron energy = 70 eV), scan range = 45-400 amu, and scan rate = 3.99 scans/sec], and Shimadzu GCMSsolution data system. The GC column was Optima-5 ms fused silica capillary with a (5% phenyl)-methylpolysiloxane stationary phase, with length of 30 m, internal diameter of 0.25 mm and film thickness of 0.25 µm. The carrier gas was helium with flow rate of 1.61 ml/min. The program used for GC oven temperature was 60 - 180°C at a rate of 10°C/min, then held at 180°C for 2 minute, followed by 180 -280°C at a rate of 15°C/min, then again held at 280°C for 4 minutes. The injection port temperature was 250°C while detector temperature was 280°C. Helium was used as a carrier gas, at a flow rate 1.61 ml/ min. Diluted sample (1/100 in hexane, v/v) of 1.0 µl was injected using autosampler and in the split mode with ratio of 10:90. The analysis was performed in triplicate. The retention indices were calculated for all volatile constituents using a homologous series of *n*-alkane under the same conditions of analysis. Individual constituents were identified by referring to compounds known in the literature, [11] and also by comparing their mass spectra with known compounds and NIST Mass Spectral Library (NIST 11). The percentages of each component are reported as raw percentages based on the total ion current without standardization. The essential oil constituents of *Garcinia kola* leaf is as detailed in Table 1.

2.4 Microbial Strains

The following microorganisms were used in the evaluation of the antibacterial activity of the essential oil: Gram-positive bacteria *Staphylococcus aureus* (ATCC 25923); Gram-negative bacteria, *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumonia* (ATCC 13883), *Escherichia coli* (ATCC 10798), fungi *Candida albicans* (ATCC No. 2876) and *Mycobacterium bovis* BCG (ATCC 35737).

2.5 Antimicrobial Activity

The minimum inhibitory concentration (MIC) values of the essential oil of *G. kola* leaf were determined in triplicate by the microdilution broth method in 96-well microplates. The oil sample was dissolved in dimethyl sulfoxide (DMSO) followed by addition of sterile Mueller-Hinton nutrient broth for bacteria and Saboraud-Dextrose nutrient broth for fungi, to achieve concentration of 400 µg/ml. The final DMSO concentration was 20% (v/v) and this solution was used as a negative control. The inoculum was adjusted for each organism to yield a cell concentration of 2×10^7 colony forming units (cfu) per ml. Ciprofloxacin (Fidson, Lagos Nigeria) was used as a positive control for bacteria and Fluconazole (Pfizer, UK) was used as the standard drug for fungi at stock concentration of 50 µg/ml. Controls of sterility for the Mueller-Hinton nutrient broth, control culture (inoculum), Ciprofloxacin, Fluconazole, essential oil and DMSO were performed. The microplates

were closed and incubated aerobically at 37°C for 24 h. The MIC values were determined as the lowest concentration of essential oil capable of inhibiting the growth of the microorganisms. All assays were carried out in triplicate. [12] Results are shown in Table 2.

2.6 Antimycobacterial Assay

Determination of antitubercular activity was carried out on *Mycobacterium bovis* BCG (ATCC 35737) using the broth dilution method previously described [13]. Mycobacterium bovis BCG cells were grown to an optical density of 0.2–0.3 at 650nm in 7H9/ ADC/Tween consisting of Middlebrook 7H9 broth supplemented with 0.5% bovine serumal bumin fraction V, 0.08% NaCl, 0.2% glucose, 0.2% glycerol and 0.05% Tween80. The essential oil sample was dissolved in dimethyl sulfoxide (DMSO), centrifuged for 20 minutes at 13,000 rpm, followed by addition of sterile 7H9/ADC/ Tween to achieve concentration of 400 µg/ml solution. The final DMSO concentration was 4% (v/v) and this solution was used as a negative control. 50 µl of media was introduced into all wells 2 to 12 of a 96-well micro-titer plate, while 100 µl of sample 400 µg/ml was delivered into the appropriate well 1 of the 96-well plate. Two-fold dilutions were performed by sequential transfer of 50 µl of each sample from well 1 to 2; after thorough mixing, 50 µl was transferred from well 2 to 3. The process was repeated through to well 11 where 50 µl was discarded leaving column 12 for the negative control. 50 µl of inoculum prepared by diluting a 5-7 day old culture of *Mycobacterium bovis* BCG (OD 0.2-0.3) 1:1000 (by adding 50 µl of cell culture into 50 ml 7H9/ADC medium) was added to all the wells and incubated for 14 days at 37°C, after which the growth or inhibition of growth was read by direct recording of visual growth. All of the minimum inhibitory concentration (MIC) determinations were done in duplicate. The MIC values were determined as the lowest concentration of essential oil capable of inhibiting the growth of the microorganisms. Isoniazid was used as positive control. Results are shown in Table 2.

3. RESULTS AND DISCUSSION

3.1 Gas Chromatography Mass Spectrometry (GCMS) Analysis

Oxygenated monoterpenes predominated in *Garcinia kola* leaf essential oil as shown in Table 1. The essential oil is mainly composed of citronellic acid (48.3%), followed by geraniol (1.52%), and menthol (0.22%). Oxygenated sesquiterpene constituents were farnesol (2.79%) and hexahydrofarnesyl acetone (2.59%). The predominant ketones were dihydropseudoionone (5.12%) and α-ionone (4.38%), with other minor constituents (3% or less of each).

The major components of *Vismia macrophylla* (Clusiaceae) leaves essential oil were beta-caryophyllene (20.1%), germacrene D (11.6%) and beta-elemene (7.0%) [14].

Table 1. Chemical composition of *Garcinia kola* leaf essential oil from Nigeria

Name	RI	% composition
Heptane, 5-ethyl-2-methyl-	927	0.84
Isobutylcyclohexane	995	0.83
Decane	1000	2.62
Mesitylene	1003	1.17
Octanal	1018	1.90
Lilac aldehyde A	1152	0.64
Geraniol	1270	1.52
Citronellic acid	1295	48.30
α-Cubebene	1347	0.72
α-Ionone	1421	4.38
Dihydropseudoionone	1451	5.12
β-Ionone	1490	0.46
α-Farnesene	1502	1.02
Farnesol	1715	2.79
n-Pentadecanol	1775	0.58
Hexahydrofarnesyl acetone	1842	2.59
Oxacycloheptadec-8-en-2-one	1918	0.63
farnesyl acetone	1920	1.70
Methyl hexadecanoate	1929	0.39
Menthol	2098	0.22
Phytol	2106	2.69
Trans-geranylgeraniol	2196	0.37
Nonacosane	2900	2.19
9-Octadecanamide, (Z)	2371	1.53
Tetracosane	2400	0.15
Squalene	2784	2.27
2-methyloctacosane	2860	0.65

RI = Kovats retention index

The major constituents identified in the leaf essential oil of *Hypericum triquetrifolium* Turra (Clusiaceae) from Calabria (Italy) were *n*-nonane (15%), *β*-pinene (4%), *α*-pinene (10%),

Table 2. Inhibitory effect on the growth of bacteria (MIC values µg/ml) of the essential oil from the leaf of *Garcinia kola*

S/No.	Microorganisms	Minimum inhibitory concentrations	
		Essential oil	Standard drug
1*	*Pseudomonas aeruginosa* (ATCC 27853)	200	48.8 ng/ml
2*	*Klebsiella pneumonia* (ATCC 13883)	50	390.6 ng/ml
3*	*Escherichia coli* (ATCC 10798)	200	390.6 ng/ml
4*	*Staphylococcus aureus* (ATCC 25923)	>400	97.7 ng/ml
5**	*Candida albicans* (ATCC No. 2876)	100	6.25 µg/ml
7***	*Mycobacterium bovis* BCG (ATCC 35737)	>400	0.07 µg/ml

*Bacterial strain; **fungal strain; ***mycobacterium strain. Ciprofloxacin (standard drug for bacterial strains); fluconazole (standard drug for fungi strain); isoniazid (standard drug for mycobacterium strain)

myrcene (5%), *β*-caryophyllene (11%), germacrene-D (13%), sabinene (3%) and caryophyllene oxide (12%) [15].

In the present study, the major constituents of essential oil from the leaf of *Garcinia kola* (Clusiaceae) were citronellic acid (48.3%), 5,9-undecadien-2-one (5.12%), α-ionone (4.38%), 3-buten-2-one (3.25%) and 2-pentadecanone (2.59%)

The most abundant constituent in the essential oil from the leaf of *Garcinia kola* was citronellic acid, a well-known acyclic monoterpene carboxylic acid. It was also reported in the essential oils of several plant species such as the *Pelargonium* species, [16] *Pelargonium graveolens*, [17] and *Eucalyptus citriodora* [18]. It is a flavor and fragrance agent [19]. It is used as an intermediate in the commercial production of *(S)-(+)-4-Methyl-3-heptanone*, the principal alarm pheromone of the leaf-cutting ant *Atta texana*; and also one of the alarm pheromones in three other ant genera of the subfamily *Myrmicinae*. *(S)-(+)-4-Methyl-3-heptanone* is a component of the defensive secretion of the "daddy longlegs" *Leiobunum vittatum* (Opiliones) and is produced by the elm bark beetles *Scolytus scolytus (F.)* and *S. multistriatus* [20]. Based on our results, the mature leaves of *Garcinia kola* from Nigeria contain essential oil characterized by citronellic acid (48.3%), 5,9-undecadien-2-one (5.12%), α-ionone (4.38%), 3-buten-2-one (3.25%), 2-pentadecanone (2.59%) and squalene (2.27%) among others.

3.2 Inhibition Activity on Microorganisms

The essential oil from the leaf of *Garcinia kola* was investigated for its potential antibacterial, antifungal and antimycobacterial activities. The oil was slightly affective for the *Escherichia coli*. Standard antibiotics ciprofloxacin, fluconazole and isoniazid showed good inhibitory action on the microorganisms tested. The oil showed significant activity against the microorganisms with the following minimum inhibitory concentrations *Staphylococcus aureus* (>400 µg/ml), *Escherichia coli* (200 µg/ml), *Klebsiella pneumonia* (50 µg/ml), *Candida albicans* (100 µg/ml), *Pseudomonas aeruginosa* (200 µg/ml) and *Mycobacterium bovis* BCG (>400 µg/ml). This supports the traditional use of *Garcinia kola* leaf for the management of tuberculosis [21]. The plant *Garcinia kola* will continue to play an important role as nutraceuticals [22].

4. CONCLUSION

Garcinia kola has been used for the treatment of diseases, such as diabetes, asthma, ulcer, infectious diseases, cancer and inflammatory conditions. The essential oil of *Garcinia kola* leaf from Nigeria was analyzed by GC-MS and was found to be dominated by citronellic acid (48.3%) followed by dihydropseudoionone (5.12%) and α-ionone (4.38%). The oil showed significant activity against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Candida albicans*, *Pseudomonas aeruginosa* and *Mycobacterium bovis* BCG which supports the folkloric use of *Garcinia kola* leaf for the management of tuberculosis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Dorcas Bolanle James, Ene Blessing Adejor, Danladi Amodu Ameh, Adetoro Olubukola Kadejo, Taiye Olaba. Preventive and therapeutic effects of *Garcinia kola* biflavonoid fractions on some haematological parameters of P407 induced hyperlipidemic albino rats. Journal of Natural Remedes. 2014;14(1):41-47.
2. Hemshekhar M, Sunitha K, Sebastin Santhosh M, Devaraja S, Kemparaju K, Vishwanatha BS, Niranjana SR, Girish KS. An overview on genus Garcinia: phytochemical aspects. Phytochemistry Review. 2011;10:325-351.
3. Russels TA. The colas of Nigeria and Cameroon. Cameroon Tropical Agriculture. 1995;32:210-240.
4. Iwu M. Handbook of African medicinal plants. CRC Press, Boca Raton, FL. 1993; 12:32-38.
5. Emmanuel O. Ogbadoyi, Adamu Y. Kabiru, Roseline F. Omotosho. Preliminary studies of the antitrypanosomal activity of *Garcinia kola* nut extract in mice infected with *Trypanosoma brucei brucei*. Journal of Medicine and Medical Sciences. 2011; 2(1):628-631.
6. Sofowora EA. The present status of knowledge of the plants used in traditional medicine in West Africa. Journal of Ethnopharmacology. 1996;47:209-264.
7. Obey Jackie, Anthoney Swamy T, Ngule Chrispus Mutuku. Preliminary phytochemical and *in vitro* control of selected pathogenic organisms by ethanolic extract of *Garcinia kola* seeds. International Journal of Current Microbiology and Applied Sciences. 2014; 3(4):183-196.
8. Oloyede OI, Durojaiye O, Adewale OB. Prevention of Fe2+ induced lipid peroxidation by aqueous extract of *Garcinia kola* leaf in some rat tissues. Innovations in Pharmaceuticals and Pharmacotherapy. 2013;1(2):128-132.
9. Nanyak Z. Galam, Ibraheem M. Gambo, Ali A. Habeeb, Ali I. Shugaba. The effect of aqueous extract of *Garcinia kola* seed on the liver histology. Journal of Natural Sciences Research. 2013;3(1):81-87.
10. Ezeanya Chinyere C, Daniel Ebakota O. Antibacterial activity of *Garcinia kola* seed and leaf extract on some selected clinical isolates. Science Journal of Microbiology. 2013;1-8.
11. Adams RP. Identification of essential oil components by gas chromatography/ quadrupole mass spectroscopy, 4th ed.; Allured Publishing Corporation: Carol Stream, IL, USA. 2007;803.
12. Okhale SE, Amupitan JO, Ndukwe G, Ayo RG, Oladosu PO, Okogun JI. Synthesis and antibacterial activity of 7-deacetoxy-7α-hydroxygedunin. African Journal of Pure and Applied Chemistry. 2013;7(4): 157-163.
13. Nneka N. Ibekwe, John B. Nvau, Peters O. Oladosu, Auwal M. Usman, Kolo Ibrahim, Helena I. Boshoff, et al. Some Nigerian anti-tuberculosis ethnomedicines: A preliminary efficacy assessment. Journal of Ethnopharmacology. 2014;155:524–532.
14. Rojas J. Buitrago A, Rojas L, Morales A. Essential oil composition of *Vismia macrophylla* leaves (Guttiferae). Natural Product Communcations. 2011;6(1):85-6.
15. Alessandra Bertoli, Francesco Menichini, Michele Mazzetti, Guido Spinelli, Ivano Morelli. Volatile constituents of the leaves and flowers of *Hypericum triquetrifolium* Turra. Flavour and Fragrance Journal. 2003;18(2):91-94.
16. Maria Lis-Balchin G. Roth. Citronellic acid: A major component in two Pelargonium Species (Geraniaceae). Journal of Essential Oil Research. 1999;11(1):83-85.
17. Farukh S. Sharopov, Hanjing Zhang, William N Setzer. Composition of geranium (*Pelargonium graveolens*) essential oil from Tajikistan. American Journal of Essential Oils and Natural Products. 2014; 2(2):13-16.
18. Nagpal N, Shah G, Arora NM, Shri R, Arya Y. Phytochemical and pharmacological aspects of Eucalyptus genus. International Journal of Pharmaceutical Sciences and Research. 2010;1(12):28-36.
19. Lu Y, Khoo TJ, Wiart C. Antioxidant activity determination of citronellal and crude extracts of *Cymbopogon citratus* by 3 different methods. Pharmacology and Pharmacy. 2014;5:395-400.
20. Dieter Enders, Helmut Kipphardt, Peter Fey. Asymmetric syntheses using the samp-/ramp-hydrazone method: (*s*)-(+)-4-methyl-3-heptanone. Organic Synthesis. 1987;65:183.

21. Omonike O. Ogbole, Edith O. Ajaiyeoba. Traditional management of tuberculosis in Ogun State of Nigeria: The practice and ethnobotanical survey. African Journal of Traditional, Complementary and Alternative Medicines. 2010;7(1):79–84.

22. Pietro Scicchitano, Matteo Cameli, Maria Maiello, Pietro Amedeo Modesti, Maria Lorenza Muiesan, Salvatore Novo, et al. Nutraceuticals and dyslipidaemia: Beyond the common therapeutics. Journal of Functional Foods. 2014;6:11-32.

Peer-review history:
The peer review history for this paper can be accessed here:
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