Phytochemical Screening of *Garcinia kola* Crude Extract and its Sensitivity Testing against *Anopheles* mosquito larva

CHEA, Sampson K. P.; DONZO, Kalilu and MULBAH, Edmund

Department of Biological Sciences, T. J. R. Faulkner College of Science and Technology

University of Liberia

e-mail: cheask@ul.edu.lr/skpchea719@gmail.com

ABSTRACT

Garcina kola is a dicotyledonous plant found predominately in the tropical rain forests and swamps, and it grows as a medium sized tree up to a height of about 12m high. It belongs to the Guttiferae Family, and it has a distinct bitter taste. It is believed to possess important source of new chemical substances with potential therapeutic benefits. This study was designed to identify the basic class of phytochemicals present in the *G. Kola* and to ascertain its Larvicidal property against the *Anopheles* mosquito larvae. The maceration technique was used to slice and pound the dried *Garcinia kola* into powder. 300g of the powder was divided into two parts, 250g and 50g respectively. The 250g was further divided into two parts by dissolving 125g each in 250 ml ethanol and water respectively. The two mixtures were then separately filtered and the filtrates were prepared as extracts of ethanol and water. These two extracts were used for the phytochemical screening. The 50g was divided into two separate parts by dissolving 25g into 25ml of ethanol and the volume made up to 100ml using water, and 25g into 100ml of water. The two stock solutions were serially diluted separately (40, 80, 120, 160, 200, 240, 280, 320 ppm), and specific quantity of Anopheles larvae were transferred into them. The larvicidal activity of both aqueous and ethanol extracts is dose dependant and it is also a function of time; the toxic effects of the crude extract on the larvae depend on the concentration and duration of exposure It can be concluded that the crude extracts of the *Garcinia kola* have phytochemicals that have medicinal benefits and insecticidal activity, particularly mosquitocidal activity, against the larvae of the *Anopheles* mosquitoes Therefore, it could be formulated and used as an ecological friendly natural product for anti-mosquito activity for the elimination and eradication of malaria.

Key words: *Garcinia kola*, Medicinal property, Secondary metabolite, Phytochemical

1.0 INTRODCUTION

Garcinia kola is a dicotyledonous plant found predominately in the tropical rain forests and swamps, and it grows as a medium sized tree up to a height of about

12m high. It is a species of flowering plants which grows in tropical climates across Western Africa, Asia, and Australia [1]. It belongs to the Guttiferae Family, it has a distinct bitter taste, hence it is commonly named “bitter kola” and “male kola” because of its claimed aphrodisiac activity, despite its bitter taste, *Garcinia* kola has been traditionally consumed for cultural, social, and traditional ceremonies.

Medicinally *G. kola* is believed to be an important source of new chemical substances with potential therapeutic benefits [2]. Bitter kola has some highly valued ingredients that made it very essential in African ethno medicine because of its varied and numerous uses which are social and medicinal, thus making the plant an essential ingredient in folk medicine

Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs [3]. Most of the world's population relies on traditional medicine for their primary healthcare needs. Plant products still remain the principal source of pharmaceutical drugs and agents [4]. Presently, there are global problems of multiple antibiotics resistance as well as emergence of new illness of previously eradicated diseases leading to a pressing need for new and more potent anti-microbial compounds of natural origin to complement the existing synthetic antimicrobial anti-sickling drugs [5].

Medicinal plants such as *Garcinia kola* had since been used traditionally as a primary source of providing health care for indigenous people, but the mixture of the various plants type create some level of side effect, besides, the known amount of chemical constituents present in each plant are unidentified and the volume of consumptions of those taking these herbs are inaccurately measured [6].

The high cost of important conventional drugs for treating diseases and/or inaccessibility to modern health care facilities has led to over reliance on traditional medicine since it is affordable. There is a need to screen these medicinal plants; understand its contents and role they play in the treatment of many diseases [7]. *Garcinia kola* is a wonder plant that contains multiple phytochemicals that are useful for medicinal purposes. It contains lavicidal property, which could be used as an insecticide to reduce the spread of anopheles mosquitos (a plasmodium carrier), a causative agent of malaria. The scope of this study focused on the qualitative analysis of the basic secondary metabolite of the *Garcinia kola* seed as well as its potential lavicidal property. This study was conducted to in order to analyse the basic class of phytochemicals present in the *G. Kola* and to ascertain the Larvicidal property of the *Garcinia kola*. The findings of this study are useful as a guide in the pharmaceutical industry for the development of new drugs against mosquito Larvae.

2.0 METHOOLOGY

2.1 Pre-Extraction phase of *Garcinia kola nuts*

This research was conducted at the Department of Biological Sciences, University of Liberia. Garcinia kola nuts were purchased on the local Liberian market in the Red-light market in Paynesville City. The kola nuts were transported in transparent polythene bags to the Laboratory for chemical identifications. They were washed with distilled water, peeled and then chopped into tiny pieces with the use of a stainless steel knife. This was done to increase the surface area needed for the sample to dry quickly. They were allowed to shadow dry for a period of one week. They were pounded in a mortal into 300g of powder; and then stored in a fridge at 30℃ until it was used for qualitative analysis.

2.2 Extraction Technique

The maceration technique was used to analyze the samples. 250g of the powder was separated into two parts (125g each) and poured into two separate conical flasks. 250ml of ethanol was poured into one of the flask and 250ml of water was poured into the other flask respectively. The 125g of *G. kola* powder dissolved in ethanol was kept for twenty four hours at room temperature (37℃), and the 125g of *Garcinia kola*

powder dissolved in water was heated for five minutes and also kept for 24 hours at room temperature. The two mixtures were then separately filtered and the filtrate was prepared as aqueous extract (222ml for the ethanol extract and 225ml for the water extract

2.3 Phytochemical Screening of Extracts

The phytochemical standard screening test was developed to identify the specific types of secondary metabolites in *Garcinia kola* sample.

2.3.1 Screening for phenolic compounds:

Phenol Test: 2ml of 1% ferric chloride was added to 2ml of aqueous and ethanol extracts in separate test tubes, with 2ml of potassium Ferro cyanide was added for the detection of phenolic compounds. The formation of a bluish-green color was taken as a positive test for phenols.

2.3.2 Screening for free Anthraquinone

Test with potassium hydroxide*:* 3ml of aqueous and ethanol extracts of Heckel were placed in separate test tubes, 5ml of 10% potassium hydroxide was added to each and stirred; a red precipitate was formed which shows positive test for Anthraquinone.

Borntrager's test: From 0.3g of Garcinia sample was added to 10ml of benzene, shaken vigorously and filtered; 5ml of 10% ammonia solution was added to the filtrate. The mixture was shaken for the second time. Pink, red or violet color was not observed in the lower phase as expected, indicating the absence of free Anthraquinone.

Test with sulfur acid*:* 0.6g of the Garcinia powdered plant sample was boiled with 10ml of concentrated sulfuric acid, and filtered when it was hot. 5ml of chloroform was added to the filtrate and shaken. The chloroform layer was pipetted and 1ml of 10% ammonia solution was added. Formation of pink, violet or red color indicated the presence of Anthraquinone.

2.3.3 Screening for Carbohydrates

Fehling's test: 2 ml of each *Garcinia kola* extract, 3 ml of Fehling's solution A and B in the ratio of 1:1 was added and the mixture boiled for 2 minutes. A brick red precipitate indicated the presence of free reducing sugar.

2.3.4 Screening for Saponins

Foam test: 3ml of each extract was dissolved in 6 ml of distilled water and then shaken vigorously for 45 seconds and allowed to stand for 30 minutes. Foam was formed for more than 20 minutes indicates saponin.

Froth emulsion test: In another test tube same procedure was carryout vigorously shaken and some drops of olive oil was added which suspended the froth from the crude solution further showing the presence of saponin.

2.3.5 Screening for Terpenoids

Three (3ml) of each extract of Garcinia kola was dissolved in 3 ml of Chloroform and 3ml of concentrated H2SO4 were added to form a lower layer. A reddish brown colour at the interface indicates the presence of terpenoids.

2.3.6 Screening for flavonoids

Shinoda test*:* Half (0.5 g) of each extract was dissolved in 2 ml of 50% methanol in the heat. Metallic magnesium and four drops of concentrated HCl were added. A red or orange colour indicates the presence of flavonoids aglycones.

Sodium hydroxide test: five drops of aqueous NaOH was added to 3 ml of each extract, a yellow colouration shows the presence of flavonoid.

2.3.7 Screening for Tanins

Ferric chloride test*:* One (1.0g) of each extract of Garcinia kola was dissolved in 10 ml of distilled water, and then filtered. Few drops of 0.1% Ferric chloride solution were added to the filtrate. Formation of a blue-black precipitate indicates tannin.

2.3.8 Screening for Alkaloids

Test for steroidal glycosides: Ten (10 ml) of each extract of Garcinia kola was evaporated to dryness in a test tube on a boiling water bath. The residue was dissolved in 1.0 ml Chloroform. The solution was transferred to a dried test tube and 4 ml of concentrated Sulfuric acid was added using a pipette from the bottom (Liebermann-Burchard's reaction). At the separating level, the two liquid were separated by a reddish brown or violet brown ring formed the superior layer being bluish green or violet for the presence of steroids and triterpenes.

2.4 Anopheles mosquito larvae screening

The balance 50g of the *G. kola* powder was divided into two separate parts (25g each). Two stock solutions were prepared as follow: firstly, by dissolving 25g of the *G. kola* powder into 25ml of ethanol and the volume made up to 100ml using water; and the secondly, by dissolving 25g of the **G kola** powder into 100ml of water. Eight different dilutions (of each mixture) of 40ppm, 80ppm, 120ppm, 160ppm, 200ppm, 240ppm, 280 and 320ppm were prepared in 300ml of deionized water in volumetric flask. Each of these concentrations was placed in 100ml beaker and specific quantity of larvae were released into each. The death of the larvae against time was taken at 1hr, 4hrs, 8hrs, 16hrs, 32hrs and 48hrs, respectively. The beakers were kept in a temperature controlled room at 28 °C ± 2 °C.

3.0 RESULTS

3.1 Phytochemical Screening

The phytochemical screening of the *Garcinia kola* aqueous extracts showed that some major secondary metabolites were present, namely, phenolic compounds, Anthraquinone, carbohydrates (reducing sugars), Saponins, terpenoids, flavonoids, Tanins, alkaloids and Steroidal compounds (see table 3.1).

Table 3.1: Phytochemical Analysis

Secondary metabolite	Ethanol Extract	Aqueous Extract
Phenolic compounds	+	+
Anthraquinone	+	-
Carbohydrates	+	+
Saponins	-	+
Terpenoids	-	+
Flavonoids	+	+
Tanins	+	+
Alkaloids	+	+
Steroidal compounds	-	+

Positive (+) shows presence; Negative (-) shows absence

3.2 Anopheles mosquito Larvae Testing

The seven different dilutions of 40ppm, 80ppm, 120ppm, 160ppm, 200ppm, 240ppm, 280 and 320ppm separately prepared in 300ml of deionized water in volumetric flask were tested against the anopheles larvae. The results are illustrated in tables 3.2 and 3.3 below:

Table 3.2: Aqueous crude extract sensitivity against Anopheles larvae

Concentration (ppm)	1hr	4hrs	8hrs	16hrs	32hrs	48hrs
40	-	-	-	-	-	-
80	-	-	-	-	-	-
120	-	-	-	+	+	+
160	-	-	+	+	+	+
200	-	+	+	+	+	+
240	+	+	+	+	+	+
280	+	+	+	+	+	+

Positive (+) shows sensitivity and Negative (-) shows no sensitivity

Table 3.3: Ethanol crude extract sensitivity against Anopheles larvae

Concentration (ppm)	1hr	4hrs	8hrs	16hrs	32hrs	48hrs
40	-	-	+	+	+	+
80	-	+	+	+	+	+
120	-	+	+	+	+	+
160	+	+	+	+	+	+
200	+	+	+	+	+	+
240	+	+	+	+	+	+
280	+	+	+	+	+	+

Positive (+) shows sensitivity and Negative (-) shows no sensitivity

Table 3.4: Mortality rate of Anopheles mosquito larvae in aqueous crude extract

Concentration (ppm)	Quantity of Larvae	Number of deaths	Percent mortality
120	60	5	8.3
160	60	15	25.0
200	60	24	40.0
240	60	33	55.0
280	60	35	58.3
320	60	60	100

Table 3.5: Mortality rate of Anopheles mosquito larvae in ethanol crude extract

Concentration (ppm)	Quantity of Larvae	Number of deaths	Percent mortality
40	60	24	40.0
80	60	29	48.3
120	60	35	58.3
160	60	32	53.3
200	60	38	63.3
240	60	44	73.3
280	60	55	91.6
320	60	60	100

4.0 DISCUSSIONS

This study was conducted to identify the phytochemical constituents or secondary metabolites of *Garcina kola* and to ascertain its larvicidal activity against the Anopheles mosquito larvae.

The phytochemicals detected in the extracts of the G. kola (Flavonoid, Saponin, Tannin, Carbohydrates, Steroidal compounds, Terpenoids, Alkaloid, Free Anthraquinone and phenolic compounds) have also been isolated from other plants [6]. These phytochemicals have been proven to have many medicinal benefits [8].

Flavonoids and other phenolic compounds have been shown to exhibit antioxidant property and they are bioactive agents found in some medicinal plants [9]. Phenolic compounds and flavonoid normally scavenge the free radicals and play an essential role in prevention and therapy of cancer, cardiovascular disease and inflammation by Inducing Anti-oxidant defense system, drug metabolizing enzymes [9]. Saponin is responsible for the reduction of blood cholesterol; by binding with bile salt and cholesterol in the intestinal tract and prevents re-absorption in the blood stream. It also contains a no sugar portion with antioxidant activity and serves to boost our immune system [10].Anthraquinone are commonly used for constipation relief by inducing water and electrolytes secretion; it is also antineoplastic [11]. Terpenoids are the primary constituents of essential oil, used as a fragrant and as well vitamin A [12]. Alkaloids are morphine and its derivatives are used as pain medication by attaching to our nerve cells and prevent the release of pain; it also contain quinine which is used to treat malaria. It also play role in neurodegenerative disorders [13]. These phytochemicals, including others that are identified in this study, are reasons for the antimalarial, anticancer, antimicrobial, and the many medicinal benefits of the *G. kola* which is regarded as a wonder plant [14].

Anti-parasitic resistance is one of a major problem which the world is facing and is resulting in increased death rate. The multiple antiparasitic resistant agents cause severe problems that result in the complication of treatment of bacterial infections and this has been recognized by the World Health Organization [15]. Anopheles mosquitoes are vectors that carry the plasmodium falciparum; which is the causative agent of malaria. Malaria is one of the world's most leading diseases with a very high death rate. The larvicidal activity of both aqueous and ethanol extracts is dose dependant and it is also a function of time; the toxic effects of the crude extract on the larvae depend on the concentration and duration of exposure [16]. At the concentration of 120ppm, sensitivity of the aqueous extract against the third and fourth instar larvae was observed after 16hrs, and as the concentration increases, the time began to decrease (see table 3.2). The lowest sensitivity was observed at 120ppm and the minimum time taken to observed sensitivity was one hour as the concentrations were increased (table 3.2). The Ethanol extract showed strong sensitivity against the *Anopheles* larvae. At a low concentration of 80ppm, after four hours, sensitivity was observed. 100% mortality was observed at 160ppm within an hour as compared to the aqueous extract which showed 100 % mortality at 240ppm within the since time. .

At the various concentrations, no lethal effect was observed until after sixteen hours and eight hours, at the 120 ppm and 40 ppm respectively (Table 3.2 & Table 3.3). The percent mortality was calculated for both aqueous and ethanol extracts (tables 3.4 & 3.5). At the concentration of 240 ppm, 55% of the larvae died in the aqueous extract while at 120 ppm and 160ppm, 58.3% and 53.3% of the larvae died in the ethanol extract respectively. This is comparable to previous reports on larvicidal activities of plant extracts. Kamaraj et al. (2011) reported LC_{50} values of 93.80 and 104.94 for the methanol extract of *Annona squamosa* leaves and methanol extract of the leaves of *Chrysanthemum indicum* L, respectively, against *Anopheles subpicus.*

5.0 CONCLUSION

The crude extracts obtained from the *Garcinia kola* contains secondary metabolites which possessed medicinal properties. It can be used as one of the source of finding chemical substances that can served as possible drug candidates for the development of new pharmaceutical agents and insecticidal agents, especially anti-mosquito agents. It can be concluded that the crude extracts of the Garcinia kola have phytochemicals that have medicinal benefits and insecticidal activity, particularly mosquitocidal activity, against the larvae of the *Anopheles* mosquitoes Therefore, it could be formulated and used as an ecological friendly natural product for anti-mosquito activity for the elimination and eradication of malaria.

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REFERENCES

[1] Farombi, O.E.(2003).African indigenous plants with chemotherapeutic potentials and biotechnological approach to the production of bioactive prophylactic agents. African Journal of Biotechnology, 2(12): 662-671.

[2] Eisner T. (1990). Chemical Prospecting. A Call for Action. In: Borman, F H and S R Keller, (Eds), Ecology, Economics and Ethics: The Broken Circle. Yale University Press, New Haven, CT, 105-110.

[3] Sofowora, A. (2006). Medicinal Plants and Traditional Medicine in Africa. Spectrum books, Nigeria, pp. 3-10. www.spectrumbooksonline.com.

[4] Ogundipe, O., Akinbiyi, O. and Moody, J. O. (1998). Antibacterial activity of isolates from Piper longunm and Taxus bakata. Pharmaceutical biology, 39: 236-238.

[5] Owumi, B. E. (1993). The place of Traditional Medicine in Primary Healthcare in Nigeria: The State of the Art. E. A. Oke and B. E. Owumi (eds). Ibadan. University of Ibadan, pp. 301-320.

[6] Rathee, S. (2012). Phytochemical screening and Antimicrobial activity of Picrorrhiza kurroa, an Indian traditional plant used to treat chronic diarrhea. Arabian Journal of Chemistry, in press

[7] Vital, P. G. and Rivera, W. L. (2009). Antimicrobial activity and cytotoxicity of *Chromolaena odorata* (L. f.) King and Robinson and *Uncaria perrottetii* (A. Rich) Merr-extracts. Journal of Medicinal Plants Research, **3(7):** 511-518.

[8] Azra Kamal and Md. Matloob Raza Khan (2014). Phytochemical evaluation of some medicinal plants; India Journal of Plant Sciences ISSN: 2319 – 3824 (online) 2014 vol 3 (4).

[9] Duangjai Tungmunnithum, Areeya Thongboonyou, Apinan Pholboon and Aujana Yangsabai (2018), Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview; (MDPI) Medicines.

[10] Eskandar Moghimipour and Somayeh Handali (2015). Saponin: Properties, Methods of Evaluation and Applications; Annual Research & Review in Biology 5(3): 207-220, 2015 Article no.ARRB.2015.022 ISSN: 2347-565X

[11] Shih-Chang Chien, Yueh-Chen Wu, Zeng-Weng Chen, and Wen-Chin Yang (2015). Naturally Occurring Anthraquinones: Chemistry and Therapeutic Potential in Autoimmune Diabetes; Hindawi Publishing Corporation Evidence-Based Complementary and Alternative Medicine: Volume 2015, Article ID 357357

[12] Vidita V. Bhargava[1*], Shashank C. Patel[1], Kruti S. Desai[1] (2013). Importance of Terpenoids and Essential Oils in Chemotaxonomic Approach. international Journal of Herbal Medicine: Volume: 1, Issue:2 First page: (14) Last page: (21) ISSN: 2321-2187

[13] Ghulam Hussain et al (2018). Role of Plant Derived Alkaloids and Their Mechanism in Neurodegenerative Disorders: International Journal of Biological Sciences 2018; 14(3): 341-357. doi: 10.7150/ijbs.23247

[14] Hosakatte Niranjana et al (2019). Evaluation of Chemical Composition and Antioxidant Activity of Cordia myxa Fruit Pulp: Journal of Herbs, Spices & Medicinal Plants Volume 25, 2019 - Issue 3

[15] WHO (2003). Fact Sheet, Traditional Medicine. Geneva, May 2003. Retrieved from www. who.int/mediacentre/factsheets/2003/fs134/en/.

[16] Sampson, K. P. CHEA and Jacob A. Kolawole (2014) " Larvicidal activity of crude extract of *Morinda morindiodes* (Jologbo) leaves on *Anopheles* mosquitoes larvae" International Journal of Biological and Chemical Sciences, Int. J. Biol. Chem. Sci., 8(2): 695-698, April, 2014