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Original article

Antimicrobial and antifungal properties of Garcinia kola on some standard laboratory pathogens

Abdulla Salih Aljabry[1*], Nagwa Malik Hussein[2], Mariam Siddig Ali [3], Leina Abdelwahab Mohamed[4], Missa Mohamed Saleh[5] and Ayat Ahmed Alrashied[6]

[1]Dean faculty of Dentistry, [2,3&4] Registrar of periodontology, [5] Head department of medical laboratories, [6] Biochemist in medical laboratory, Faculty of Dentistry, University of Medical Sciences and Technology- Khartoum- Sudan.

ABSTRACT

Introduction: In different African and Middle East countries, people used to chew Garcinia kola(G.K) seeds driven mainly by beliefs of traditional backgrounds. Recently, G.K was researched for its prospected antimicrobial benefits. **Aims**: To study the antimicrobial effects of G.K on some standard laboratory bacteria and fungi. **Objectives:** To test the effect of different concentrations of water extracts and ethanol extracts of G.K. on four bacteria and two fungi; namely Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans and Aspergillus niger. **Materials and methods**: Seeds of Garcinia cola were obtained from the local market. Samples were extracted by cold extraction technique using ethanol and water. The paper disc diffusion method was adopted with some minor modifications to assess the antibacterial activity of the prepared extracts. **Results**: All water and ethanol extracts of G.K, showed antimicrobial activity. **Conclusion:** G.K. has antimicrobial activity against the different bacteria and fungi tested. Activity was measured by the width of the inhibitory zone which varied with solvent and concentration of the active substance**.**

KEYWORDS: Abdalla Aljabry; Garcinia kola, Pseudomonas aeruginosa, Candida albicans and Aspergillus niger.

INTRODUCTION

Garcinia kola, traditionally known as GORO, is well known plant which is grown in West Africa. It is the nut of the Kola tree (a member of the family Sterculiaceae); it is a genus of trees native to the tropical rainforest of Africa. It is mostly produced in West Africa and is cultivated to a large degree in Nigeria, but also in Ghana, Ivory Coast, Brazil and the West Indian Islands. The seeds of this plant are chewed for its believed medical benefits. It has social, cultural and religious significance [1],[2],[3],[4],[5].

There are over 125 species of kola nut trees, two species Cola nitida and Cola acuminata are of major economic importance.

There is strong belief that Bitter cola can treat many diseases namely diabetes, hypertension, gout, impotence and many other diseases. On the other hand, there is strong belief that it can improve the general health. However, there is no published data about the use of Bitter cola in Sudan although it is very common practice in many communities. Recently some global data clarifying the antimicrobial effects of Bitter cola became available. It is proved by some researchers that the aqueous extract of the seeds of this plant has bactericidal properties [6],[7].Other authors concluded that Bitter cola also has potent antifungal properties.

Chemical composition: Different species of cola nut (cola nitida, cola acuminata and Garcinia cola) have proximate comparable properties:

1. Moisture content 20.62 - 22.50%
2. Ash 2.50 - 3.00%
3. Crude proteins 8.65 - 8.70%
4. Carbohydrate 61.11 64.05%
5. Crude fat 0.80 - 0.90%
6. Crude fiber 3.38 - 4.25%
7. Caffeine content 2.42 - 2.96%

Uses and benefits: cola nut contains two alkaloids; caffeine and theobromine, which are powerful stimulants that counteract fatigue, suppress thirst and hunger. Kola nut also help increase oxygen levels in the blood and promote better concentration and clearing of the head. Traditionally, the leaves, twigs, flowers, fruits follicles, and the bark of both C. nitida and C. acuminata were used to prepare a tonic as a remedy for dysentery, coughs, diarrhea, vomiting, headache, and chest complaints [8] Help promote digestion. Bitter kola improves lung functions, used as remedy for osteoarthritis, malaria and glaucoma.

Kolaviron content of bitter kola exhibit many pharmacological effects such as antioxidant, antiatherogenic, antihepatotoxic, antidiabetic and have a cardioprotective effect. Garcinia kola has an antifungal, antimicrobial and anticariogenic effects. It is used as "chewing sticks" during fasting to clean the teeth and gums and to freshen breath. It is used chiefly for flavoring cola drinks such as Coca-Cola. It is also offered in ceremonial rituals where the nut is considered a symbol of hospitality and kindness.

In Nigeria, Amalu Paul C. et al studied the antimicrobial effects of Garcinia cola against some microbial organisms, namely: Staphylococcus aureus, Escherichia coli and Candida albicans. The results of their study showed that the seed of Garcinia kola had some degree of inhibitory effects on Staphylococcus aureus (Gram +ve) and E. coli (Gram – ve), with no inhibitory effect on Candida albicans. [8]. In another study by Adejare O. Y

Et al[9] concluded that the extract of Garcinia kola possesses anticandidal activity and provide preliminary evidence of the presence of one or more soluble constituents with antifungal activity and pointed to the possibility of using this cheap plant as a potential source of oral rinse. V. Kuete et al. [11] also studied the potential properties of Garcinia kola against some bacteria and candida and concluded that results of their study provide an important basis for the use of methanolic extract from *G. smeathmannii* for the treatment of infections associated to the microorganisms used in this study.

The crude extract as well as the isolated compounds found active in this study could be useful for the development of new antimicrobial drugs. However, further pharmacological and toxicity studies will be necessary to confirm these hypotheses. Nwaokorie et al.[12] study demonstrated that extracts obtained from Garcinia kola displays good activity against clinical isolate of F,nuclatum and is associated with periodontal pathogens the reduction of population of F.bacterium nucleatum during the experiment can be related to the inhibitory activity produced by the extract thus the extract may be an alternative for maintaining oral hygiene.

Objectives:

1. To study the effect of water and ethanol extracts of Bitter cola on some periodontal pathogens.
2. To identify the most sensitive and resistant microorganism to Bitter cola extract.

MATERIALS AND METHODS

Extraction of plant: seeds of Garcinia cola were obtained from the local market. The seeds were firstly pealed, then grounded and dried. Samples were extracted by cold extraction technique using ethanol and water according to the required concentrations.

The test organisms: four reference strains of bacteria and two fungi were kindly provided by Stack laboratory- Sudan. The laboratory work was conducted at Sudan Central laboratory, Khartoum between the periods April to May/ 2017.

Reference strains of bacteria and fungi:

Bacillus subtilis	NCTC 8239	Gram positive rods
Staphylococcus aureus	ATCC 25923	Gram positive cocci
Escherichia coli	ATCC 25922	Gram negative rods
Pseudomonas aeruginosa	ATCC 27853	Gram negative rods
Candida albicans	ATCC 7596	Yeast fungi
Aspergillus niger	ATCC 9763	Filamentous fungi

ATCC is the American Type Culture Collection, Rockville, Maryland, U.S.A.**NCTC** National Collection of Type Culture, Colindale, England.

Culture media*:*

Nutrient broth: this medium contains peptone, yeast extract and sodium chloride. It was prepared according to Barrow and Fellthan, 1993[9] by dissolving 13 gram of the medium in one litre of distilled water. The pH of the medium was adjusted to 7.4 and the medium was then distributed into screw capped bottles, 5 ml each and sterilized by autoclave at 121°C for 15 minutes.

Mueller Hinton agar*:* thirty eight grams of the powder of Mueller Hinton agar were weighed, dissolved in 1 litre of distilled water and allowed to soak for 10 minutes. The medium was placed in water bath to dissolve, swirled to mix and sterilized by autoclaving for 15 minutes at 121°C, cooled to 47°C, mixed well then poured into sterile Petri dishes.

Sabouraud Dextrose agar: sixty two grams of the powdered Sabouraud dextrose agar was weighed, dispersed in 1 litre water and allowed to soak for 10 minutes, swirled to mix then sterilized by autoclaving for 15 minutes at 121 °C, cooled to 47 °C, mixed well then poured in to sterile Petri dishes.

Assay for antibacterial activity:

Disc diffusion method: when a filter paper disc impregnated with a chemical is placed on agar the chemical will diffuse from the disc into the agar. This diffusion will place the chemical in the agar only around the disc. The solubility of the chemical and its molecular size will determine the size of the area of chemical infiltration around the disc. If an organism is placed on the agar it will not grow in the area around the disc if it is susceptible to the chemical. This area of no growth around the disc is known as a "zone of inhibition". The paper disc diffusion method of Kil et al, 2009 [9] was adopted with some minor modifications to assess the antibacterial activity of the prepared extracts.

Twenty ml aliquots of the molten Mueller Hinton agar were distributed into sterile Petri-dishes. About 0.1 ml of the standardized bacterial stock suspension 108 –109 C.F.U/ ml were streaked on Mueller Hinton agar medium plates using sterile cotton swab. Sterilized filter paper disc (6 mm diameter) were soaked in the prepared extracts, and then were placed on surface of the test bacteria plates. The plates were incubated for 24 h and the diameters of the inhibition zones were measured. Reference drugs and 10% Dimethyl sulfoxide (DMSO) were used as the positive and negative controls, respectively. After incubation period, the diameters of the resultant growth inhibition zone were measured. Mean and standard error values were tabulated.

Bioassay for antifungal activity: The same method described for bacteria was adopted to test the antifungal activity, Sabouraud Dextrose Agar was used. The inoculated medium was incubated at 25˚C for two days for the Candida albicans and three days for Aspergillus niger.

RESULTS

The results of this study show that Garcinia Kola possesses antibacterial and antifungal properties against the tested microorganisms (Table 1). This antibacterial and antifungal activity varied according to the concentration of the water or ethanol extract (Table 2). The most effective ethanol concentration was 5mg/ml for the *Bacillus subtilis*, 20mg/ml for *Staphylococcus aureus*, 5mg/ml for *Escherichia coli*, 5mg/ml for *Pseudomonas aeruginosa*, and 20mg/ml for *Candida albicans* and20mg/ml for *Aspergillus niger. These* results were statistically significant (Table 3).

Table 1: Anti-microbial activity of extracts against four bacterial and two fungal strains

Sample	Conc.	Bacteria strain				Fungi strain	
		B.s	*S.a*	*E.c*	*P.a*	*C.a*	*A.n*
Ethanol extraction	20 mg/ml	11±0.0mm	13±1.4	9±0.0	12±0.0	13±0.0	12±0.7
	10 mg/ml	11±0.7mm	11±0.7	10±0.7	10±0.0	12±0.0	9±0.0
	5 mg/ml	13±0.0mm	11±0.7	12±1.4	15±0.0	12±0.0	11±0.0
Water extraction	20 mg/ml	15±0.0mm	10±0.0	10±0.7	15±1.4	13±0.0	14±0.0
	10 mg/ml	8±1.4mm	12±0.7	10±0.0	21±0.7	13±0.0	10±0.0
	5 mg/ml	11±0.0mm	12±0.7	11±0.0	10±0.0	11±0.0	11±0.7
Standard	10mg/ml	15±0.7mm	15±0.0	19±0.0	17±0.7	13±0.7	15±0.0

B.s = Bacillus subtilis, S.a = Staphylococcus aureus, E.c = Escherichia coli, P.a = *Pseudomonas aeruginosa*, *C.a = Candida albicans, A.n = Aspergillus niger*. Interpretation of results: MDIZ* (mm): < 9 mm = Inactive, 9-12 mm = partially active, 13-18mm = Active, >18 mm = Very active. *M. D. I. Z = Mean diameter of growth inhibition zone in mm.

Table 2: shows the most effective concentration of solvent compared to standard.

Bacterium	Most effective conc. of solvent	Standard inhibition
Bacillus subtilis	20 mg/ml water = 15±0.0	15±0.7
Staphylococcus aureus	20 mg/ml ethanol =13±1.4	15±0.0
Escherichia coli	5 mg/ml ethanol = 12±1.4	19±0.0
Pseudomonas aeruginosa	10 mg/ml water = 21±0.7	17±0.7
Candida albicans	20 mg/ml ethanol and10 mg/ml water = 13±0.0	13±0.7
Aspergillus niger	20 mg/ml water = 14±0.0	15±0.0

The most effective water concentration was 20mg/ml for *Bacillus subtilis*, 10 and 5mg concentrations showed the same effect on *Staphylococcus aureus*, 5mg/ml is more effective against *Escherichia coli* and10mg/ml is the effective concentration against *Pseudomonas aeruginosa.* *These* results were statistically significant (Table 3). Regarding the tested fungal species, the most effective concentration against *Candida albicans* and *Aspergillus niger* was 20mg/ml of both ethanol and water ,this is statistically significant.

Table 3: The effect of water and ethanol extract on four bacteria.

	Treatment	N	Mean	Std. Deviation	Std. Error	F-Test	P-Value
B.s	20 mg/ml-Ethanol extract	2	15.00**	0.000	0.000	28.278	0.00*
	10 mg/ml-Ethanol extract	2	8.00*	1.414	1.000		
	5 mg/ml-Ethanol extract	2	11.00*	.000	.000		
	20 mg/ml-Water extract	2	11.00*	.000	.000		
	10 mg/ml-Water extract	2	10.50*	.707	.500		
	5 mg/ml-Water extract	2	13.00**	.000	.000		
	Standard	2	14.50	.707	.500		
	Total	14	11.86	2.413	.645		
S.a	20 mg/ml-Ethanol extract	2	10.00*	.000	.000	10.750	0.00*
	10 mg/ml-Ethanol extract	2	11.50*	.707	.500		
	5 mg/ml-Ethanol extract	2	11.50*	.707	.500		
	20 mg/ml-Water extract	2	13.00**	1.414	1.000		
	10 mg/ml-Water extract	2	10.50*	.707	.500		
	5 mg/ml-Water extract	2	10.50*	.707	.500		
	Standard	2	15.00	.000	.000		
	Total	14	11.71	1.773	.474		
E.c	20 mg/ml-Ethanol extract	2	9.50**	.707	.500	1.574	0.28**
	10 mg/ml-Ethanol extract	2	16.00**	8.485	6.000		
	5 mg/ml-Ethanol extract	2	10.50*	.707	.500		
	20 mg/ml-Water extract	2	10.50**	2.121	1.500		
	10 mg/ml-Water extract	2	10.00**	.000	.000		
	5 mg/ml-Water extract	2	13.00**	2.828	2.000		
	Standard	2	17.50	2.121	1.500		
	Total	14	12.43	4.033	1.078		
P.a	20 mg/ml-Ethanol extract	2	15.00**	1.414	1.000	74.944	0.00*
	10 mg/ml-Ethanol extract	2	21.50*	.707	.500		
	5 mg/ml-Ethanol extract	2	10.00*	.000	.000		
	20 mg/ml-Water extract	2	12.00*	.000	.000		
	10 mg/ml-Water extract	2	10.00*	.000	.000		

	5 mg/ml-Water extract	2	15.00**	.000	.000		
	Standard	2	15.50	.707	.500		
	Total	14	14.14	3.880	1.037		

*Significant different at the 0.05 level, **Not significant different at the 0.05 level.
B.s = Bacillus subtilis, S.a = Staphylococcus aureus, E.c = Escherichia coli, P.a = *Pseudomonas aeruginosa, C.a = Candida albicans, A.n = Aspergillus niger*.

DISCUSSION

The results of this study indicate that there is antimicrobial activity of Garcinia kola against the tested pathogens. The activity varies according to the type of solvent and the concentration used (Table 1).the concentrations used varied between 5, 10, and 20mg/ml dissolved in either water or ethanol. All concentrations showed zones of inhibition however in some cases, lower concentrations showed more inhibition zone than higher ones as in the case of 5m/ml ethanol which is more potent against *Escherichia coli* than higher concentrations.

These results agree with the conclusions made by Amalu Paul C1 et al [8] their previous study however; Candida albicans is proved sensitive to 20mg/ml ethanol and 10mg/ml water G.K. extracts respectively. This is in contrary to the results of Amalu Paul C1 et al. [8] but it agrees with the results of Adejare O. Y Et al who concluded that the extract of Garcinia kola possesses anticandidal activity and provide preliminary evidence of the presence of one or more soluble constituents with antifungal activity and pointed to the possibility of using this cheap plant as a potential source of oral rinse. V. Kuete et al. [11] also confirmed the potential activity of Garcinia kola against some bacteria and Candida and concluded that results of their study provide an important basis for the use of methanolic extract from *G. smeathmannii* for the treatment of infections associated to the microorganisms used in this study.

The crude extract as well as the isolated compounds found active in this study could be useful for the development of new antimicrobial drugs. However, further pharmacological and toxicity studies will be necessary to confirm these hypotheses. Nwaokorie et al. [12] again affirmed that extracts obtained from Garcinia kola display good activity against clinical isolate of F. nucleatum and the reduction of population of F.bacterium nucleatum during the experiment can be related to the inhibitory activity produced by the extract thus the extract may be an alternative for maintaining oral hygiene.

CONCLUSION

The results of this study cast additional lights on the antimicrobial activity of Garcinia kola against the tested microorganisms. All concentration [water and ethanol] were markedly effective. It is also noticed that sometimes low concentration are more effective than higher ones e.g. 5mg/ml ethanol is more effective than 10, 15 and 20mg/ml.

All cultures showed considerable inhibitory zones especially the Candida albicans which constitutes a major health problem in early childhood in underdeveloped countries; so it is necessary to recommend further studies on this issue due to the potential pharmaceutical benefits of Garcinia kola.

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*Corresponding author: Abdalla Saleh Aljabry
E-Mail: abdugab2@yahoo.com