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Original Research

Phytochemical and *in-vitro* antimicrobial properties of *n*-butanol seed extract of *Garcinia kola*

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Abstract

Objective: Phytochemical and *in vitro* antimicrobial activity of the *n*-butanol seed extract of *Garcinia kola* was investigated against some bacterial isolates.

Methods: Six (6) bacterial isolates of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Salmonella. typhi*, *E. coli* and *P. aeroginosa* were use to investigate the anti bacterial activity, minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC).

Results: The phytochemical analysis of the *n*-butanol seed extract indicates the presence of carbohydrates, alkaloids, flavonoids, cardiac glycosides, and tannins. The extract exhibited significant inhibitory action against *Streptococcus pyogenes*, *Staphylococcus aureus* and *Bacillus subtilis*. The zone of inhibition exhibited by the extract against the tested organisms ranged between 7 and 19 mm while zone of inhibition exhibited by tetracycline as the standard antibiotics ranged between 19 and 32 mm. On the other hand, MIC against the bacterial isolates (*Streptococcus pyogenes, Staphylococcus aureus* and *Bacillus subtilis*) was 100 and 200 mg/ml, while MBC was 100, 200 mg/ml.

Conclusion: The *n*-butanol extract of *Garcinia kola* has antibacterial activity hence validating the folkloric claims and application of the decoction of *Garcinia kola* in the management of some bacterial infections especially of the oral cavity and dental caries by traditionalists and herbalists in Africa especially in Nigeria.

INTRODUCTION

Medicinal plants have for generation been used for the treatment of ailments including infectious diseases such as typhoid, tuberculosis, colibacillosis, aspergillosis, ulcer and many other infections that has defied orthodox therapy. The problem of bacterial resistance exhibited by *Staphylococcus aureus* to commonly used antibiotics and high cost of conventional drugs particularly in poor countries of the developing world has necessitated the search for newer plant based drugs and alternative compounds for the treatment of drug resistant infections [1]. *Garcinia kola* grows in evergreen forests found in moist semi-deciduous forest zones and savannah. It is cultivated and distributed throughout west and central Africa where it is valued for its medicinal properties [2, 3].

Garcinia kola is used in folklore medicine in many tropical countries to fight infectious diseases such as AIDS, Ebola and for the treatment of ailments such as liver disorders, hepatitis and diarrhoea. The seeds are commonly used by communities for the treatment of headache bronchitis, throat infections and also to prevent and relieve colic, cure head or chest cold and relieve cough. The stems and twigs of the plant are used as chewing sticks in maintenance of oral hygiene [5,16,10].

The objective of this study is to evaluate the phytochemical, antimicrobial properties, minimum inhibitory and bactericidal concentration of *Garcinia kola* on some susceptible microbial organisms.

MATERIALS AND METHODS

Sample collection, identification and extract preparation

The seeds of *Garcinia kola* were obtained from kola nut dealers at Monday market, Maiduguri, Borno state, Nigeria, and were botanically identified and confirmed by Prof. S. S Sanusi of the Department of Biological Sciences, University of Maiduguri, Nigeria, and Boucher specimen was deposited at the herbarium of the same Department. The seeds were dehusked, air-dried and ground into powder. Two hundred and fifty grams (250 g) of the powder was mixed with 2.5 L of distilled water (25°C) in a beaker and the mixture was shaken vigorously. Shaking was repeated after every 30 min for the next 6 h before it was allowed to stand for 18 h. thereafter it was shaken vigorously before was filtered using Whatman No. 1 filter paper. The filtrate was dried in an oven (DHG-9030A) at 50 °C and stored in a glass container at 4 °C until required.

Phytochemical screening of *Garcinia kola*

The aqueous extract of *Garcinia kola* was subjected to quantitative chemical screening for the identification of the various classes of active chemical constituents. The Phytochemical analysis was carried out according to the method described by [11, 13, 9].

Culture media

Nutrient agar and Nutrient broth (Oxoid, England) were used for the investigation.

Preparation of microbial culture

Laboratory isolates of *Streptococcus pyogenes, Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae, Salmonella typhi, Pseudomonas aeroginosa, Escherichia coli,* were obtained from the Department of Veterinary Medicine laboratory, University of Maiduguri, Nigeria. The isolates were cultured separately on nutrient agar plate and incubated for 24 h. The medium (25 ml) was poured unto sterile petri dish and allowed to solidify. A colony of each test organism was sub cultured on 10 ml nutrient broth and incubated at 37°C for 8 h. One millilitre of the broth culture was used to flood the agar plate.

Preparation of inocula

The inoculum size of all bacterial isolates tested was standardized by the use of overnight broth cultures prepared by inoculating 3 loopfuls of well- isolated colonies of test bacteria in 10 ml of Nutrient broth which was incubated at 35°C for 24 hours. A loopfuls of the overnight broth culture was diluted in 4ml of sterile physiological saline (0.8% W/V) such that its turbidity marched with that of 0.5 Mac Farland standard (a barium sulphate standard) considered to have a mean bacterial density of 3.3 x 10^6 CFU /ml. This was gauged by comparing the turbidity of the test suspension with the turbidity 1% (W/V) Barium sulphate solution against the background of a printed white paper [8]

Preparation of antimicrobial discs

Graded concentrations of 200, 400, 600, 800 and 1000 mg / ml of the extract were measured and poured into different plates. About 1ml of sterile distilled water was added to each plate containing the extract and stirred. Filter paper discs (6mm) diameter were then placed in each plate and stirred so as to ensure the impregnation of the disc by the extract. 250 mg/ml Tetracycline as the control drug was prepared the same way as the extract and placed at the centre of each inoculated plate. Plates containing fungal isolates mixed with extract were also prepared.

Preparation and impregnation of paper disc with *n*-butanol seed extract of *Garcinia kola*.

Paper discs (6mm in diameter) were punched from No. 1 Whatman filter paper using an office puncher. These were used to prepare discs which were impregnated with five different concentrations 200, 400, 600, 800 and 1000 mg/ml of the extract. 250 mg Tetracycline was used to impregnate control discs. Plates containing fungal isolates mixed with extract were also prepared.

Incubation of bacterial and fungal isolates

The inoculated plates containing filter paper discs (6 mm) impregnated with the extract and control drug were incubated at 37°C for 18-24 hours. The plates containing fungal isolates mixed with extract were also kept at room temperature for 96 hours and observed for growth.

Minimum inhibitory concentration (MIC)

The MIC of the *n*-butanol seed extract of *Garcinia kola* was determined using the method of Greenwood [15] as described by Geidam [14]. Serial dilution of the extract was done to obtain the concentrations of 200, 100, 50, 25 and 12.5 mg/ml respectively and used to determine MIC. MIC was recorded as the least concentration of the extract that completely inhibited the growth of the organisms.

Minimum bactericidal concentration (MBC)

The MBC of the extract was determined using the method of Geidam [14]. Samples were taken from test tubes in the MIC assay and sub-cultured unto freshly prepared nutrient agar medium and later incubated at 37°C for 24hrs. The MBC was taken as the lowest concentration of the extract that inhibits bacterial growth on the agar plates.

RESULTS

The Phytochemical analysis of the aqueous extract of *Garcinia kola* revealed the presence of carbohydrate alkaloids, flavonoids, cardiac glycoside and tannin while anthraquinones, steroids and tannins were not detected in the extract (Table 1). The *n*-butanol seed extract possesses antimicrobial activities against *Streptococcus pyogenes, Staphylococcus aureus Bacillus subtilis*, *Klebsiella pneumonia* and *Salmonella* typhi, at graded concentrations of (400, 600, 800, and 1000 mg/ml) but showed resistance to *E*. coli, and *Pseudomonas* aeroginosa, Tetracycline (250 mg/ml) which was used as the control drug inhibited the growth of all the micro-organisms including those resistant to the *n*-butanol seed extract of *Garcinia kola*. (Table 2). The MIC test showed that *Streptococcus pyogenes*, *Staphylococcus aureus* and *Bacillus subtilis* were inhibited at 100 and 200 mg/ml respectively (Table 3). The MBC inhabited the growth of *Streptococcus pyogenes*, *Staphylococcus aureus* and *Bacillus subtilis* at concentration of 100 and 200 mg/ml. Chronologically *Streptococcus pyogenes* is most susceptible organism to the extract followed by *Staphylococcus aureus* and *Bacillus subtilis*. (Table 4).

Table 1. Phytochemical analysis of the *n*-butanol seed extract of *Garcinia kola*

Phytochemical constituents	Test	Inference
Tannin	Ferric chloride	+
	Lead acetate	+
	Hydrochloric acid test	-
Saponin	Frothing	-
	Fehling	+
Alkaloid	Dragendurff's	-
	Mayer's	-
Carbohydrate	Molisch's	+
	Barfoed's	++
	Combined reducing sugar	+++
	Free reducing sugar	+++
	Ketoses	+
	Pentoses	-
Phlobatannins	Hydrochloric acid	-
Cardiac glycosides	Salkowski's	+++
	Liabermann– Burchard's	+
	Terpenoids	++
Steroid	Liaberman's	-
	Salkowski's	-
Flavonoids	Shinoda's	+++
	Ferric chloride	+
	Sodium hydroxide	+
	Lead acetate	+
Anthraquinones	Free anthraquinones	-
	Combined anthraquinones	-

KEY: (+) Present, (-) Absent

Table 2. Antibacterial activity of *n*-butanol seed extract of *Garcinia kola* on some bacterial organisms

Extract / Antibiotic	Amount of extract & tetracycline (mg)	Zone of inhibition diameter(mm)						
		Organisms						
		S aureus	Strept. Pyogenes	*B. subtilis*	*K. Pneumoniae*	Salm. typhi	*E. coli*	P. aeroginosa
	1000	13	14	10	12	11	R	R
	800	11	11	9	10	9	R	R
	600	19	9	8	8	7	R	R
	400	7	7	9	R	R	R	R
	200	R	R	9	R	R	R	R
Tetracycline	250	19	22	32	17	20	28	23

KEY: (R) Resistant

Table 3. Determination of minimum inhibitory concentration (MIC) *n*-butanol seed extract of *Garcinia kola*

Organisms	Concentration of *Garcinia kola* aqueous seed extract (mg/ml)				
	200	100	50	25	12.5
Strept pyogenes	-	-	+	+	+
Staph aureus	-	-	+	+	+
Bacillus subtilis	-	-	+	+	+

KEY: + Growth observed
- Growth inhibited

Table 4. Determination of minimum bactericidal concentration (MBC) of *Garcinia kola* aqueous seed extract

Micro- organisms	Concentration of *Garcinia kola* aqueous seed extract (mg/ml)				
	200	100	50	25	12.5
Strept. pyogenes	-	-	+	+	+
Staph. aureus	-	-	+	+	+
Bacillus subtilis	-	+	+	+	+

KEY: + Growth observed
- Growth inhibited

DISCUSSION

For many years, medicine depended exclusively on leaves, flowers and barks of plants, only recently have synthetic drugs come into use and in many instance, these are carbon copies of chemical identified in plants. In orthodox medicine, a plant may be subjected to several chemical processes before its active ingredients are extracted, while in traditional medicine, a plant is simply eaten raw, cooked or infused in water or native wine or even prepared as food [4].

Naturally occurring substance of plant origin have been reported to inhibit the growth of microorganisms. Plants extracts have also been used in folk and even modern medical practices for the treatment of different ailments, most of which are due to microbial activities. Bacterial infections seem especially controllable due to good hygiene and the availability of effective antibacterial drugs. The development of resistance to antibiotics is an almost inevitable consequence of their application [14]. The speed of resistance depends on the respective class of antibiotics and their product use.

The phytochemical screening and quantitative estimation of the aqueous extract and the chemical constituent of the plant studied showed that the extract was rich in carbohydrate alkaloids, flavonoids, cardiac glycoside and saponins. These components have been known to show medicinal activities as well as physiological activities [11]. The plant studied can be seen as potential source of useful drug. On the other hand, *n*-butanol extracts of *G. kola* showed a wider spectrum of activity. But the plant showed greater activity on *Staphylococcus aureus, Salmonella typhi, Staphylococcus pyogenes, Klebsiella pneumoniae,* and *Bacillus subtilis.* It can be deduced that *n*-butanol *G. kola* may be used to fight opportunistic infections caused by these organisms. The inactivity exhibited by *Garcinia kola* against*, Pseudomonas aeroginosa, Escherichia coli,* may perhaps be due to the absence of inhibitory alkaloids against the organisms. This is because alkaloids have been claimed to be responsible for antimicrobial effect [4, 6]. However, the above reason may not be strong enough to justify that no any other bioactive agent present in the extract. This is similar to the report of Geidam [14] showed that no active substance exhibited its maximum activity under laboratory experimental conditions. Therefore, activity may be recorded if greater concentrations are used. In addition, ingredients of the media [7], pH size and inoculum [12] may be attributed to the inactivity of some of the extracts.

CONCLUSION

G. kola ranked well among the medicinal plants used routinely among many tribes in Nigeria and some part of Africa for the treatment of infections caused by microorganisms. Apart from been used for folklore remedies, *G. kola* seeds are also chewed by many people because of their bitter taste. These suggest that the seeds are not toxic and hence there is need for the preparation of different formulations towards ensuring acceptable dosing regimen pursuant to clinical trials. Further work should be carried out on the mechanisms of action of the plant.

REFERENCES

1. Uniyal SK, Singh KN, Jammal P, Lal B. Traditional use of medicinal plants among the tribal communities of Chhota, Bhengal. Western Himalaya. Journal of Ethnobiology and Ethnomedicine 2006; 2:1-14.
2. Okojie AK. Phytochemistry and Preliminary toxicity studies of the methanol extract of the stem bark of *Garcinia kola.* Int J Toxicol 2009; 7(2):118-20.
3. Sibanda T, Okoh AI. *Invitro* antibacterial regimes of crude aqueous and acetone extracts of *Garcinia kola* seeds. J Biol Sci 2008; 8(1):149-54.
4. Waller GR, Nowaki ER. Alkaloids production and metabolism in plants. Plenum Press, New York, pp 56-60, 1978.
5. Ayensu ES. Medicinal plants of West Africa, Reference Publications Inc, Algonac, Michigan, p 162, 1978.
6. Lehane B. The power of plant. Oxford University Press, Oxford, pp 45-48, 1977.
7. Bevallius B, Zacharias N. Applied Microbiology in Clinical Bacteriology, 5th edition, pp 72-79, 1971.
8. Cheesbrough M. District Laboratory Practices in Tropical Countries Part 2. Cambridge. University Press, Cambridge, pp 137-139, 2002.
9. Edeoga HO, Okwu DE, Mbaedie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotechnol 2005; 4(7):605-88.
10. Farombi EO, Tahteng JG, Agboola AO, Nwankwo JO, Emerole GO. Chemoprevention of 2-acetylammoflourene induced hepatotoxicity and lipid peroxidation in rats by kolaviron-a *Garcinia kola* seed extract. J Food Chemical Toxicol 2000; 38(6):535-41.
11. Sofowora A. Screening plants for bioactive agents. In: Medicinal plants and traditional medicine in Africa. 2nd (Edn). Spectrum Books LTD. Sunshine House, Ibadan, pp 81-93, 134-156, 1993.
12. Stokes EJ, Ridway GL. Clinical Bacteriology, 5th edition Arnold Publishers, London, pp 145-148, 1980.
13. Trease GE, Evans WC. Textbook of Pharmacognosy. 12th (Edn). Bailliere Tindall, London, pp 21- 22, 1983.
14. Geidam AY, Ambali AG, Onyeyili PA. Phytochemical screening and antibacterial properties of organic solvent of fraction of *Psidium guajava* aqueous leaf extracts. Int J Pharmacol 2007; 3:68-73.
15. Greenwood D. Antibiotic sensitivity testing. In: Antimicrobial chemotherapy. Greenwood (Edn). Oxford University Press, New York, USA, pp 91-100, 1989.
16. Iwu MM, Duncan R, Okunji CO. New antimicrobial of plant origin. ASHS press, Alexandria, VA, USA, pp 57-462, 1999.