



## ANTIBACTERIAL ACTIVITY OF *GARCINIA KOLA* AND *COLA NITIDA* SEED EXTRACTS

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### ABSTRACT

**Antibacterial activity of *Garcinia kola* (Bitter Kola) and *Cola nitida* (Kola nut) against *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli* and *Klebsiella pneumoniae* was studied. Results showed that alcohol extract of *Garcinia kola* was active against *Staphylococcus aureus* and *Klebsiella pneumoniae* at various concentrations, with the latter displaying the lowest sensitivity. *Escherichia coli* and *Salmonella typhi* were completely resistant. Hot water extract of the same plant was however, active against *Salmonella typhi*, *Staphylococcus aureus* and *Klebsiella pneumoniae*. The other organisms showed sensitivity to the alcohol extract of *Cola nitida*, but higher sensitivity was observed with the hot water extract of the plant. Some of the results provided scientific evidence for the use of the plants by traditional herbalists in the treatment of microbial infections.**

**Key word: *Garcinia kola*, Bitter Kola, *Cola nitida*, Kola nut, Antibacterial, extracts.**

### INTRODUCTION

*Garcinia kola* (Bitter kola), "Namijin Goro" in Hausa (Dalziel, 1916; Mshui, 1981): "Orogbo" in Yoruba; "Adili" in Ibo, is a member of the family *Guttiferae*. It is also commonly called false or male kola (Dalziel, 1948). It is a tree plant that is commonly found in tropical West African countries such as Nigeria and Sierra Leone (Mukhtar and Shuaibu, 1999), which grows as a medium size tree, up to 12m high. The tree has straight bole with somewhat drooping branches (Iwu *et al.*, 1999). Fruits are produced from July to October. They are subglobose, reddish yellow and about 2.5inches in diameter, containing 2-4 ellipsoid, brown seeds 3 – 3.5cm long. 1.5cm broad embedded in an orange – coloured pulp (Keay *et al.*, 1964, Mukhtar and Shuaibu, 1999). The seeds are the important products. They do not separate into two cotyledons. They have, in chewing a bitter astringent and resinous taste, somewhat resembling that of the raw coffee bean, with a residual slight sweetness. The seeds are chewed as stimulants among the native (Hutchinson and Dalziel, 1954). Medicinally, the seeds are said to prevent or relieve colic and particularly they are curative for colds in the head or chest, relieving cough and hoarseness and improving the singing voice (Dalziel, 1948). Other medicinal uses of the plant include, its use as an anti-hypertensive, in the treatment of urinary tract infections, and as an aphrodisiac, it is also used in the treatment of liver disorders and as a chewing stick (Walter and Lewis, 1977).

*Cola nitida* (Kola nut) "Goro" in Hausa; "Obi gbanja" in Yoruba; "Oji" in Ibo (Keay *et al.*, 1964) is a member of the family *Steculicca*. It is a tree plant found in Sierra Leone. North Ashanti, tropical Western Africa, West Indies, Brazil and Java, which grows about 40 feet high (Grieve, 2001). The tree has unbranched bole for several feet; narrow buttress extending up bole for 3 feet in old trees and also has

grey bark with longitudinal fissures. Fruits are produced from September to January and June to July. They are composed of up to 5 carpels borne on a short handing stalk, the carpels usually in recurved position; carpels are green and shiny, smooth to the touch, but knobby with large warts and a pronounced keel which extends into the short curved beak, up to 5.5 inches long and 3 broad, seeds up to 10, covered with a white skin, usually with only 2 cotyledons (Keay *et al.*, 1964). Fresh kola nut seeds which resemble conkers, consist of a large percentage of water. Observation taken at Moor plantation in southern Nigeria on freshly harvested nuts indicated that 60 to 70% of the nut weight is water (Eijnatten, 1996). The phytochemical composition of the kola nut seed is of obvious interest, since it is reported to cure so many ailments. The key components are caffeine, theobromine, tannins, phenolics, phlobaphene, anthocyanin, pigment kola red, betaine, protein and starch (Karcher, 1997). The medicinal uses of kola nut are recognized officially indicated as toxic, stimulant, laxative, sedative and diuretic (Eijnatten, 1966).

In line with the generally accepted methods of searching for biological active substances from plants (Deeni and Hussain, 1991; Fatope *et al.*, 1993) the research was aimed at screening for *in-vitro* antibacterial activity of the methanolic and water soluble extracts of seeds of *Garcinia kola* and *Cola nitida* against four clinical bacterial isolates.

### MATERIALS AND METHODS

#### Sample collection and processing

The seeds of *Garcinia kola* and *Cola nitida* were obtained from kolanut dealers at Kurmi Market, Kano city, Nigeria. These were botanically identified and confirmed at the herbarium of the Department of Biological Sciences, Bayero University, Kano, Nigeria with the aid of literature (Gbile, 1980). The seeds were dehusked, air-dried and ground into powder.

**Extraction procedure**

**Methanol extract**

Air – dried and ground seeds (100g) of the plant were extracted with 95% aqueous methanol (500ml) in the ratio of 1:5 (w/v) at room temperature overnight. The mixture was filtered and the filtrate was evaporated to dryness and stored in a deep freezer until needed for the test (Aishamma and Mitscher, 1979).

**Aqueous extract**

Water extracts were obtained by mixing the powdered seed materials of the plant (100g) with distilled water (500ml) at 70°C in a water bath for 2 hours. They were filtered and the filtrates were evaporated to dryness, stored in clean bottles and placed in a deep freezer for further use (Fatope *et al.*, 1993).

**Test organisms**

The organisms used for the test were *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Salmonella typhi* and *Escherichia coli*. The isolates were obtained from the Murtala Muhammad Specialist Hospital, Kano and the Infectious Diseases Hospital (IDH), Kano, Nigeria as pure clinical isolates and were maintained on fresh Nutrient agar slants.

**Culture Media**

Nutrient agar and Nutrient broth (oxoid) were used for the investigation.

**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 10ml 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<sup>6</sup> 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 ( Cheesbrough, 2002 )

**Preparation and impregnation of paper disc with seed Extracts of *Garcinia kola* and *Cola nitida***

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 (500µg,

1000µg, 1500µg, 2000µg and 2500µg).Ampiclox was used to impregnate control discs.

**Bioassay procedure**

Agar diffusion method as described by Kirby – Bauer (1966) and outlined by WHO (1983) was employed. The nutrient agar plates were dried in drier for 15 minutes at 45°C to remove excess surface moisture. The plates were each seeded evenly with 1ml suspension of the inoculum.

Control discs and Impregnated sterile discs were placed (five per plate) and pressed firmly on to the agar surface. The plates were incubated aerobically at 37°C for 24 hours. Diameters of the zones of inhibition were recorded in millimeters for each concentration of the active fractions. 00 indicates negative and no effect, diameter ≤ 8.0mm (zone of inhibition) indicates low activity, >8mm of inhibition indicates high activity.

**RESULTS**

Tables 1 – 4 show the results for antibacterial activity of the extracts (methanol and water fractions) of the seeds of *Garcinia kola* and *Cola nitida*. Methanol soluble extract of *Garcinia kola* showed no activity against *Escherichia coli* and *Salmonella typhi*. But it showed a moderate activity against *Klebsiella pneumoniae* at a concentration range of 1500 - 2500µg.

Water soluble extract of *Garcinia kola* showed no activity against *E. coli* (Table 2). Activity against both *Staphylococcus aureus* and *Klebsiella pneumoniae* was shown at a concentration range of 2000 - 2500µg. The extract was active against *Salmonella typhi* at all concentrations used.

Methanol soluble extracts of *Cola nitida* showed activity against both *Escherichia coli* and *Klebsiella pneumoniae* at a concentration range of 1500 - 2500µg (Table 3). It exhibited activity against *Staphylococcus aureus* and *Salmonella typhi* both at a concentration range of 2000 - 2500µg.

Water soluble extract of *Cola nitida* exhibited activity against *Escherichia coli* at all concentrations used (Table 4). Activity against *Staphylococcus aureus* was observed at a concentration range of 2000 - 2500µg, activity against *Salmonella typhi* was at a concentration range of 1000 - 2500µg, while that against *Klebsiella pneumoniae* was at a concentration range of 1500 - 2500µg.

**Table 1: Antibacterial activity of methanol soluble extract of *Garcinia kola***

Test organism	Concentration of extract (µg/ml)				
	500	1000	1500	2000	2500
	Zone of inhibition (mm)				
<i>Escherichia coli</i>	00	00	00	00	00
<i>Staphylococcus aureus</i>	17.5	18	18.5	20	21
<i>Salmonella typhi</i>	00	00	00	00	00
<i>Klebsiella pneumoniae</i>	00	00	15	18	24

**Table 2: Antibacterial activity of water soluble extract of *Garcinia kola***

Test organism	Concentration of extract (µg/ml)					
	500		1000	1500	2000	2500
Zone of inhibition (mm)						
<i>Escherichia coli</i>	00	00	00	00	00	00
<i>Staphylococcus aureus</i>	00	00	00	07	15	15
<i>Salmonella typhi</i>	11	13	14	15	20	20
<i>Klebsiella pneumoniae</i>	00	00	00	07	10	10

**Table 3: Antibacterial activity of methanol soluble extract of *Garcinia kola***

Test organism	Concentration of extract (µg/ml)					
	500		1000	1500	2000	2500
Zone of inhibition (mm)						
<i>Escherichia coli</i>	00	00	07	11	20	20
<i>Staphylococcus aureus</i>	00	00	00	12	25	25
<i>Salmonella typhi</i>	00	00	00	21	24	24
<i>Klebsiella pneumoniae</i>	00	00	07	11	20	20

**Table 4: Antibacterial activity of methanol soluble extract of *Cola nitida***

Test organism	Concentration of extract (µg/ml)					
	500		1000	1500	2000	2500
Zone of inhibition (mm)						
<i>Escherichia coli</i>	13.5	15	18	21	23	23
<i>Staphylococcus aureus</i>	00	00	00	07	09	09
<i>Salmonella typhi</i>	00	11	13	14	18.5	18.5
<i>Klebsiella pneumoniae</i>	00	00	09	11	12.5	12.5

## DISCUSSION

Antimicrobially active components of *Garcinia kola* have been successfully extracted in petroleum ether, ethanol, methanol and water. The activity of the agents against both Gram positive and Gram negative organisms was reported to be due to the presence of a poly-iso-phenyl benzophenone (kolanone) and ethyl acetate (Maduban, 1995). Its great inflammatory action was also evaluated and was found to be very similar to that of salicylic acid (aspirin) and or phenyl butazone inflammatory responses. Perhaps it may be efficacious in sore throat, boil and pneumonia due to *Klebsiella pneumoniae* and the likes.

Investigation on *Cola nitida* was also carried out. It was found to contain caffeine, theobromine and kolatine. In Nigeria, various concoctions are made from roots, seeds and leaves obtained from a variety of the kola tree. They are administered orally as purgatives, as direct cures or preventions of all sorts of diseases (e.g. leprosy, dysentery, colic, hemorrhoids, malaria fever and jaundice) (Andah, 1992; Dalziel, 1948).

The present investigation confirms, therefore, the antimicrobial activity of extracts of *Garcinia kola* and *Cola nitida*. The methanol extracts of *Garcinia kola* showed a wider spectrum of activity than the water extract. Thus, *Garcinia kola* may be of value in reducing the high incidence of Enterobacterial and urinary tract infections especially in Northern Nigeria. It may also be of value in treating typhoid fever.

Water soluble extracts of *Cola nitida* on the other hand showed a wider spectrum of activity than the methanol extract. But the plant showed greater activity on *Staphylococcus aureus* when extracted with methanol than when extracted with water. From the *in-vitro* study, it can be deduced that *Cola nitida* may

be used to fight opportunistic infections caused by *Escherichia coli*.

The inactivity exhibited by *Garcinia kola* against *E. coli* and *S. typhi* 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 (Walter and Nowaki, 1978; Lehane, 1977). 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 Burger (1990) who 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 (Bevallius and Zacharias, 1971), pH size and inoculum (Stokes and Ridway, 1980) may be attributed to the inactivity of some of the extracts.

## CONCLUSION

Methanol and water soluble fractions of *Garcinia kola* and *Cola nitida* possess antibacterial activity, *G. kola* was more active against some members of Enterobacteriaceae, namely, *Escherichia coli* and *Salmonella typhi*, whereas, methanol extracts of *Cola nitida* showed greater activity on *S. aureus*. Thus, the plants possess potentials for the manufacture of potent drugs for the treatment of infections caused by the test organisms, such as typhoid fever, gastroenteritis, urogenital tract infections and boils.

## RECOMMENDATION

From the findings of this study, it is recommended that these plants should be studied further in order to determine their pharmacological principles.

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