

# Antibacterial Effects of *Garcia Kola* Seeds Extracts on Some Selected Clinical Isolates

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**Abstract**—This is antibacterial effect of aqueous ethanol extract of seeds of bitter kola (*Garcia kola*) carried out using well agar diffusion technique. The ethanol extract of the seeds showed higher inhibitory activity on all the tested organisms as compared to aqueous extract. The results showed that salmonella typhi have high zone of inhibition of 25mm followed by Klebsiella spp 20mm, E. coli 16mm, staphylococcus aureus 15mm and Proteus spp 12mm at 1.0% concentration of the *Garcia kola* ethanol extract. The extract was found to show bacteriostasis effect on the tested organisms. Finding suggests shows the possible use of the seed of *Garcia kola* against the organisms that show in vitro susceptibility. Further study may be carried out to ascertain the efficacy, toxicity and suitability of using the extracts in vivo.

**Keyword:** *Garcia kola*, bacteriostasis, antibacterial effect, in vivo.

## 1. INTRODUCTION

Multi-drug resistance by various bacterial infections against the most commonly prescribed antibiotics is a course for concern among both medical practitioners, pharmaceutical industries, research institutes and the general populace. This has resulted in many people in both the urban and rural areas to seek for succor in plants search for cure to infections such as respiratory tract, stomach disorders and other bacterial infections. In any case, about 80% of the remedies for several diseases of the local population are cheap, readily available and safer alternative sources as antibacterial agents (Bibita et al., 2002).

*Garcia kola* has many names. In English it is called Male kola, in Hausa Namijin goro, in French Petit kola and Orogbo kola in Yoruba.

*Garcia kola* (Bitter kola) seeds are served in Nigeria and other West African countries to guest as supplement to true kola.

The pharmacology extracts of stems, roots and seeds has shown strong anti-hepatotoxic and hepatotropic activity (Iwu, 1984).

## Aims

The aims of this research project are to screen the ethanolic and aqueous extract of the seeds.

## Objectives

Determination of antibacterial activity against four (4) strains of the following pathogenic bacteria including *staphylococcus aureus*, *Klebsiella spp*, *Proteus spp* and *Salmonella typhi*.

## 2. METHODOLOGY

### 2.1 Collection of plant material

The *Garcia kola* (Bitter kola) seeds were obtained from Mariri market ('Yangoro) along Maiduguri road, Kano city. The plant material (seeds) was identified and verified by in the Biological Sciences Department of Bayero University, Kano, Nigeria.

### 2.2 Sample preparation

The thin-sliced samples of *G. kola* were washed with distilled water and dried in an oven at 50°C for 24 hours. The samples were pulverized into fine powder using mortar and pestle (Chikeze, 2007).

### 2.3 Extraction and fractionation

*G. kola* crude extract, 40g of powdered was extracted with 500ml of petroleum ether using soxlet apparatus for 12hours for the removal of fat soluble components. The sediments were dried, weighed and extracted with 500ml of ethanol using same apparatus and same time.

Ethanol was evaporated using rotary evaporator at less than 400°C while the aqueous extracts of the plant material were prepared by adding 10g sample into 100ml of distilled water. The suspension was allowed to stand for 24 hours at room temperature and filtered with Whatman No.1 filter paper.

The filtrates constituted the stock aqueous of the plant specimen (10% w/v) and serial dilution were made to obtain corresponding aqueous extract concentration in the order 1.0%, 0.8%, 0.6%, 0.4% and 0.2% (w/v) (Chikeze, 2007).

## 2.4 Preparation of culture media (NA)

The culture media were prepared by dissolving 14g of nutrient agar (NA) in 500ml distilled water. The solutions were then sterilized in an autoclave at 121°C for 15 minutes.

## 2.5 Preparation of nutrient broth

7g of nutrient broth powder was dissolved in 500ml of distilled water and stirred well to mix completely. This was then dispensed in 10ml each in a test tube and sterilized in autoclave at 121°C.

## 2.6 Collection of test organisms

The test organisms used in the antibacterial assay were clinical isolates obtained from the microbiology / pathology department of Murtala Muhammad Specialist Hospital, Kano. The identification of these organisms to the species level was done through biochemical tests and growth in differential media and selective media.

## 2.7 Preparation of standard inoculum

2 – 3 colonies of the clinical isolates were picked and inoculated into 10ml of nutrient broth in test tubes. These were then incubated at 37°C for 18 hours in ambient air as demonstrated by Mukhtar and Okafor (2002). The resulting turbid broth culture was then standardized to the turbidity of 0.5 McFarland (A 625 = 0.09) using 8.5% (w/v) physiological saline (NaCl) as the diluents (Iwalokun et al., 2001).

## 2.8 Bioassay procedure

The well agar diffusion technique was employed as demonstrated by (Stoke, 1975) this bioassay procedure.

Punch-hole method was used to measure the zone of inhibition. 12 petri dishes were poured with already sterilized nutrient agar to the level of obtaining a standard and allowed to set.

1ml each of the standard inoculums was poured into set petri dishes and uniform distribution was ensured. Sterile cork borer of 4mm in diameter was used to punch-holes in the agar. Each hole (numbering 6) per plate were filled with extracts. (1.0%, 0.8%, 0.6%, 0.4% and 0.2%) control hole was filled with solvent and allowed to stand for 30 minutes (pre-diffusion period), then kept in an incubator for 24 hours at 37°C for the organisms to grow.

## 2.9 Measurement of zone of inhibition

The zone of inhibition diameter of semi confluent growth was measured with the aid of a meter rule to the nearest mm with respect to each isolate and concentration. The keys followed as demonstrated by Mukhtar and Okafor (2002) were employed:

0mm

no effect.

Diameter < 8.0mm

(zone of inhibition) indicates low sensitivity.

Diameter > 8.0mm

(zone of inhibition) indicates high sensitivity.

## 3. RESULTS

**Table I: Antibacterial activity of ethanolic extract of *G. kola* seed against five (5) different clinical isolates.**

Test organism control	Concentration of extract (%)					Negative control
	1.0%	0.8%	0.6%	0.4%	0.2%	
	Zone of inhibition (mm)					
<i>Staphylococcus aureus</i>	15	10	00	00	00	00
<i>Escherichia coli</i>	16	11	10	10	05	00
<i>Klebsiella spp</i>	20	15	11	10	05	00
<i>Proteus spp</i>	12	10	11	00	00	00
<i>Salmonella typhi</i>	25	20	15	10	05	00

**Table II: Antibacterial activity of aqueous extract of *G. kola* seed against five (5) different clinical isolates.**

Test organism control	Concentration of extract (%)					Negative control
	1.0%	0.8%	0.6%	0.4%	0.2%	
	Zone of inhibition (mm)					
<i>Staphylococcus aureus</i>	10	05	00	00	00	00
<i>Escherichia coli</i>	07	07	00	00	00	00
<i>Klebsiella spp</i>	10	05	00	00	00	00
<i>Proteus spp</i>	10	05	00	00	00	00
<i>Salmonella typhi</i>	17	10	00	10	00	00

The extracts of the plant were tested for antibacterial activity at various concentrations on some selected isolates. *Salmonella typhi* was found to be highly sensitive to ethanolic extract at 1.0% but moderately sensitive to aqueous extracts of the plant at the same percentage. *Klebsiella spp* was also found to be highly sensitive to ethanolic extracts at 1.0% aqueous extracts.

## 4. DISCUSSION

In spite of the spectacular advances made in the field of synthetic organic drugs, interest in the microbiology, chemistry and pharmacology of medicinal plants and plant products has increased tremendously in recent years. This is probably because almost 80% of present day medicines of the world are directly or indirectly obtained from plants (Mayers, 1982).

This increasing interest is being constantly demonstrated by frequent occurrence of national and international conferences, workshops and seminars on medicinal plants.

According to Farns and Bengel (1977), this large quantity of modern drugs comes from less than 15 % of the plants which are known to have been investigated pharmacologically. One of the most intensive areas of natural products research is the extraction of useful bioactive agents from plants. However, only 10 % of all plants had been investigated for bioactivity (Sofowora, 1993).

*Staphylococcus aureus* was also found to be highly sensitive to ethanolic than aqueous extract at different concentrations.

*Escherichia coli* was also sensitive to 10 % ethanolic and aqueous extracts but resistant to all other concentrations.

## 5. RECOMMENDATION

Looking at the general research study of the seeds of *Garcia kola* for the nutritional and antibacterial activity, further study is required on the pharmokinetics of the plant's extracts and the mode of administration to ascertain the efficacy, toxicity and suitability of using the extract *in vivo*.

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