

PHYTOCHEMICAL ANALYSES AND ANTI-MICROBIAL ACTIVITIES OF THE LEAF AND STEM BARK EXTRACTS OF *GARCINIA KOLA* - HERKEL (FAMILY GULTIFERAE)

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Preliminary phytochemical analyses carried out on the leaves and stem bark extracts of *Garcinia kola* revealed the presence of glycosides, saponins, tannins and flavonoids. Alkaloids were present only in the leaf extract. Thin layer chromatography (TLC) on silica gel using different solvent systems showed the alkaloids to be both of salt and basic forms. Different concentrations of methanolic extracts (50µg/ml, 100µg/ml, 150µg/ml and 200µg/ml) were tested on cultures of bacteria and fungi to determine their sensitivity and minimum inhibitory concentrations (MIC). Antibiotic gentamycin (1mg/ml) was used as the standard drug. The stem bark and leaf extracts of *Garcinia kola* had very high inhibitory activities, only on *Staphylococcus aureus* with MIC of 100µg/ml and 150µg/ml, respectively. The stem bark and leaves of this plant can be used for the treatment of diseases caused by *Staphylococcus aureus*.

The use of plants as medicines is due to the presence of secondary metabolites in form of alkaloids, saponins, glycosides, anthraquinones and volatile oils which may be present in the roots, barks, stems, fruits, flowers leaves and seeds of plants has promoted their use as medicines, (Gill 1992). *Garcinia kola* commonly known as bitter kola. It is a plant indigenous to the West and Central African sub - regions. It is easily recognised, by its fine, hairy flowers and large fruits. It has numerous pharmacological effects. Fresh fruit of *Garcinia kola* is used as a food; hot water extracts of the dried fruits are used in treating arthritis, liver disease, and cough; while the dried fruit is used as an antiseptic for cuts, sore throats and laryngitis, (Iwu and Igboko 1982). The hot water extract is also used in treating asthma, dysmenorrhoea, gastroenteritis, diarrhoea,

hepatitis, abdominal colic and as a general antidote (Elujoba 1995).

The bark of *Garcinia kola* is used for fever cough, as an anti-helminthic and for respiratory disease while the hot water extract has anti-oxidant activity (Ebana *et al* 1991). The alkaloid fractions of the bark has spasmolytic activity, while the ethanolic extracts and glycoside mixtures have antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumonia*, *Streptococci* and antifungal activity against *Aspergillus spp.* (Braide 1989; Madunyi 1995).

This study investigated the phytochemical constituents of the stem bark and leaves of *Garcinia kola* and evaluated the anti-microbial activities of their extracts on some human isolates from a reference hospital in Benin City, Nigeria.

Plant material. The fresh leaves of *Garcinia kola* were collected from Okodobor village, near Benin City, while the stem bark was collected from Iyowa village close to Okada town, Benin City, Nigeria. They were identified by, Alhaji Abubakara taxonomist. The leaves were dried in an oven at a temperature of 40-50°C and ground into fine powder with the aid of a grinding machine. The bark was chopped into bits and pieces and also dried in the oven at 40-50°C for two weeks. It was then ground into powder. Both the stem bark and leaf powders were stored in clean dry containers at 5°C until needed.

Preparation of extracts. *Leaves* One hundred gram of powdered *Garcinia kola* leaves were extracted with one litre of methanol using the Soxhlet extractor and then concentrated with the aid of the rotary evaporator. Twenty-two gram of the methanolic extract was dissolved in 22 ml of distilled water (w/v).

Stem bark: Two hundred gram of powdered stem bark of sample was put into 800 ml of water and macerated for two days with occasional stirring. It was then filtered and the filtrate concentrated over a water bath. Approximately, 4.85g of stem bark extract was obtained.

Phytochemical analysis: Extracts were subjected to phytochemical analysis to detect glycosides, flavonoids, tannins, saponins and alkaloids using the standard analytical procedures (Shoppe 1964; Harborne 1973; Trease and Evans 1983). Thin layer chromatography (TLC) using silica gel as adsorbent and different solvent systems and as well as Dragendorff's spray reagent were used to the alkaloidal spots.

Antimicrobial activity: Agar diffusion assay technique of Tramer and Fowler was used. Autoclaved nutrient agar medium was immediately place in a 55°C water bath. When cooled, 18ml aliquots of the medium were aseptically poured

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Table 1
Phytochemical analyses of the stem bark and leaf extracts of *Garcinia kola*

Compound	Leaf Extract	Stem Bark Extract
Glycosides	Present	Present
Flavonoids	"	"
Tanins	"	"
Saponins	Absent	Absent
Alkaloids	Present	Absent

into round, plastic petri dishes and allowed to solidify and cool on room temperature. Then, excess moisture on the surface of the plates was evaporated in an incubator (30°C) for 24 h. Six wells were punched in the solidified medium using a sterile glass tube (6 mm diameter). The disks of agar were removed from the plate using a vacuum device. Crude extracts of the samples were diluted to the desired concentrations and were immediately added into the wells in the agar plates, in triplicates and allowed to solidify. A soft agar overlay consisting of 10⁴ CFU/ml of the appropriate test culture and 5ml of molten nutrient agar was poured over the surface of the plates. Plates were then incubated for 24 h at 37°C, and the diameters of the inhibition zones were measured using a vernier caliper. Mean diameters of triplicate inhibition zones were calculated. Standard drug was gentamycin antibiotics (1mg/ml), which was already in solution. The tests organisms used were clinical isolates obtained from a reference hospital in Benin City, Nigeria and included; *Staphylococcus aureus*, *Klebsiella pneumonia* *Escherichia coli*, *Pseudomonas aeruginosa*, *Aspergillus niger* and *Geotrichum species*.

The impregnated paper disc method described Casal 1979 and Chung *et al* 1990 was also used to determine the antimicrobial activities of the two extracts. The minimum inhibitory concentrations (MIC) of both the stem bark and leaf extracts were also determined by using varying concentrations of the extracts and testing using the method already described.

Phytochemical tests carried out on the leaves of *Garcinia kola* revealed the presence of glycosides, saponins, flavonoids, alkaloids and tannins. Thin layer chromatography (TLC) on silica gel using different solvent systems (methanol, water and ammonia, Benzene/Methanol showed the alkaloid to be both of salt and basic forms.

The stem bark also revealed the presence of glycosides, flavanoids and tannins. Alkaloids were absent (Table 1). Antimicrobial properties of plants have been linked to the presence of glycosides, saponins, tannins, terpenes alkaloids, unsaturated terpenes and steroids, especially tannins (Leven *et al* 1979; Hashem *et al* 1980). All test organisms except *Staphylococcus aureus* showed no activity. Table 2 also shows the antibacterial activity of the leaf and stem bark extracts.

Plants are made up of primary and secondary metabolites. These metabolites in most cases have medicinal values, (Iwu and Anyanwu 1982). Results from the phytochemical analyses of the stem bark and leaves of *Garcinia kola* have revealed the presence of flavonoids, tannins and glycosides. Saponins and alkaloids were detected only from the stem bark and the alkaloids were present in both the basic and salt forms.

The microbiological evaluations of the stem bark extract showed that the aqueous extract only had inhibitory activity against *Staphylococcus aureus* at all concentrations used, while there was no activity on the other test organisms. This showed the extract to be highly potent but very limited in its spectrum of activity. The MIC of the aqueous extract of the stem bark was found to be 100mg/ml on *Staphylococcus aureus*.

For the leaf extract, the result only showed inhibitory activity on *Staphylococcus aureus* using methanolic solvents. The minimum inhibitory concentration of the extract on *Staphylococcus aureus* was 150mg/ml. *Garcinia kola* is well used in traditional medicine for various ailments and diseases and can be very readily used in the treatment of diseases caused by *Staphylococcus aureus*.

Table 2
Zones of Inhibition of *Garcinia kola* leaf and stem bark extracts after 24hr on *Staphylococcus aureus*.

Concentration of Extract (Leaves) (mg/ml)	Zone of Inhibition (mm)	Concentration of Extract (Stem bark) (mg/ml)	Zone of Inhibition (mm)
100	-	100	10.1
150	12	150	10.2
200	19	200	11.8
Gentamycin (1mg/ml)	32	Gentamycin (1mg/ml)	20.1

- indicating no activity

Key words: Phytochemical, Antimicrobial activity, Leaf, Stem bark, *Garcinia kola*.

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