

## The effects of Diethyl ether and Aqueous *Garcinia kola* seeds extracts on some bacterial isolates.

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**Abstract:** The antibacterial activity of *Garcinia Kola* seeds Diethyl ether and Aqueous extracts were tested against selected clinical bacterial isolates; *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* at 50, 100, 150mg/ml. Agar well diffusion method was employed to determine the Minimum Inhibitory Concentration (mm) of the extracts against test isolates. The results showed that Diethyl ether exhibit more significant activity at  $P < 0.01$  and Aqueous extracts at  $P < 0.05$ , at the different treatment regimes. Similarly, the concentration of 150mg/ml produced the highest zone of inhibition (mm), while, 50mg/ml gave the least against the test isolates. Also, phytochemical compounds (Flavonoids, tannin, Saponins, Sterols, and terpenes) possibly responsible for the antibacterial activity in the plant extracts were determined. Implication of results is that Diethyl ether and Aqueous *Garcinia kola* seed extracts possess strong antibacterial and potentially chemotherapeutic activity and it can be useful in the treatment of bacterial infections in humans.

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### Introduction:

*Garcinia kola* popularly referred to as bitter kola is an indigenous medicinal tree belonging to the family Clusiaceae (formally Guttiferae). It is known as Orogbo in Yoruba land, Namijin-goro among the Hausas, Akuilu in Igbo land. It is cultivated and distributed throughout West and Central Africa and is known mostly for its antimicrobial potentials (Natural Standard Monograph, 2008). It is well branched evergreen and grown as a medium sized tree mostly about 12m high in 12 years and sometimes up to 28m height (Akerle, et al, 2007).

*Garcinia kola* is divided into different species depending on their geographical distribution and location viz; *Garcinia kola* Heckel, *G. Courauana*, *G. Cambogia*, *G. bracteaeta*, *G. mangostana*, *G. multiflora*, *G. neglecta*, *G. puat*, *G. pyrifer*, *G. actroviridis*, e.t.c. (Natural Standard Monograph, 2008).

The species which is one of the most important trees valued in Nigeria, for its medicinal seeds is *Garcinia kola* Heckel. The bark of the tree is thick and broadly elliptic, acute or shortly acuminate at the apex. The fruits are reddish yellow and about 6cm in diameter with 2-4 brown seeds embedded in an orange colored pulp (Keay, et al, 1989).

*Garcinia kola* has been used for medicinal purposes in West Africa, most especially Nigeria, for many years. As a whole, the tree has been referred to as a "wonder plant" because almost every part of it has been found to be of medicinal importance

(Oguntola, 2008). The seeds have pharmacological uses in treating cough, throat infections, bronchitis, hepatitis, liver disorders (Farombi, et al, 2005). Other medicinal uses include laxative, antiparasitic, antibacterial, antiviral and antimicrobial properties (Ebana, et al, 1991; Akoachere, et al, 2002; Anegebe, et al, 2006). The plant also found usefulness in the treatment of stomachache and gastritis (Ajebesone and Aina, 2004).

The seed of the *Garcinia kola* constituents includes; biflavonoid, xanthones, alkaloids, benzophenones (Anegebe, et al, 2006) and Saponin (Nwokeke, 2008). The flavonoid are non-toxic and can be found in orange and lemon rinds as well as the colorings of other plants (Oguntola, 2008). The chemical Saponin is mainly used as tonic for the liver. It enhances the functions of the liver and gall bladder (Nwokeke, 2008). The antimicrobial properties of the seeds are attributed to the benzophenones and flavonoids (Anegebe, et al, 2006).

### MATERIALS AND METHODS:

#### Plant Materials

The seeds of *Garcinia kola* used in this study were purchased from Wunti Market Bauchi, the Capital of Bauchi State, Nigeria. The sample was brought to the herbarium of biological sciences department of Abubakar Tafawa Balewa University Bauchi, for identification and authentication.

#### Preparation of the seed extracts

*Garcinia kola* seeds were dried at room temperature and were ground into fine powder with pestle and mortar. The powdered seeds was extracted using Soxhlet method of extraction with Diethyl ether and Aqueous extracts using cold extraction method with sterile distilled water respectively as described by Obi and Onuoha (2000), Akerele,etal(2007). The extracts was allowed to evaporate to dryness using rotary evaporator and the dried extracts were stored in an air tight colored bottle protected from Sunlight until needed for the analysis.

#### **Treatment of the seed extracts**

A concentration of 50mg/ml, 100mg/ml, and 150mg/ml respectively of the dried extract was freshly prepare for the antimicrobial activity by dissolving the extract in sterile distilled water ( Ntiejumokwu and Onuwukaeme,1991,Ogbulie,et al,2004).

#### **Test Organisms**

The organisms used in this study were confirmed clinical bacterial isolates;*Staphylococcus aureus*, *Escherichia coli*,and *Pseudomonas aeruginosa*. These were collected from the Microbiology laboratory, State Specialist Hospital,Bauchi. The isolates each was sub-cultured on nutrient agar slants and stored at 4°C until required for the analysis.

#### **Antibacterial assay**

The agar well diffusion method was used to determine the inhibitory effects of the seed extracts against the test isolates;*Staphylococcus aureus*,*Escherichia coli*, and *Pseudomonas aeruginosa* as described by ( Ntiejumokwu and Alemike,1991;Ogueke,et al,2006). The bacterial isolates were grown in nutrient broth for 18hrs at 37°C before use. The isolates were aseptically cultured onto nutrient agar medium which has been allowed to dry. The inoculated nutrient agar medium was bored using a sterilised cork borer (6mm in diameter). The wells were then filled up with the solution of the extracts at 50,100,and 150mg/ml concentration respectively and care was taken not to allow the solution to spill to the surface of the medium. The plates were then allowed to stand on the laboratory flat bench for one hour to allow proper in flow of the solution into the medium. The plates were incubated in an incubator at 37°C for 24hrs. The plates were later observed after 24hrs of incubation for the zones of inhibition,which was measured in millimeter diameter,using meter rule (Adegboye,et al,2008).

#### **Determination of Minimum Inhibitory Concentrations ( MICS)**

#### **of Diethyl ether *Garcinia kola* seed extract and Aqueous *Garcinia kola* seed extract on the selected clinical isolates.**

The MICs of the extracts was determined using the agar dilution method of Akinpelu and Kolawole (2004), Akerele,et al,(2007) respectively. Two fold dilution of the plant extract was prepared and the extract was incorporated into molte nutrient agar at concentration of 150,100,50,25,12.5,6.25,3.125 and 1.56mg/ml aseptically,mixed gently and was allowed to solidified. The surface of the agar plates were allowed to dry properly before inoculating with the bacterial isolates and the plates were then incubated at 37°C for 72hrs,after which the plates were examined for the presence or absence of growth. The lowest concentration preventing the bacterial growth in each determination was taken as the Minimum Inhibitory Concentration.

#### **Statistical Analysis**

This result was subjected to two way Analysis of Variance (ANOVA) using the randomized complete block design. The response of the bacterial isolates to the different extracts at various concentrations of the treatment regimes;50,100,150mg/ml and the mean of the zones of inhibition were determined.

#### **Phytochemical Screening Tannins**

Three grams of the seeds powder was boiled in 50ml of sterile distilled water for 3minutes on a hot plate. The mixture was filtered and the resulting filtrate was used to carried out the test for tannin using the ferric chloride method. A portion of the aqueous extract was diluted with sterile distilled water in a ratio of 1:4 and a few drop of 10% ferric chloride solution was added. A blue or green color indicates the presence of tannins (Evan,1989).

#### **Saponins**

The Froth was used to carried out this test. To a small quantity of the seed powder was added 95% ehanol and boiled. The mixture was allowed to cold and filtered. Then 2.5ml of the filtrate was added to 10mls of distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 30seconds. Honey cumb froth is indictaive of the presence of Saponins (Sofowora,1993).

#### **Flavonoids**

The sodium hydroxide method was used to carried out this test. Five grams of the seeds powder was completely de tanned with acetone. The residue was extracted with warm water after evaporating the acetone on a water bath. The mixture was filtered and the filtrate was used for the test ( Segelmmann,et

al,1971). About 5ml of 10% sodium hydroxide was added to an equal volume of the de tanned water extract. A yellow solution indicates the presence of Flavonoids.

### Alkaloids

A portion of ten grams of the powdered seed was taken in a small beaker and a strong solution of ammonia solution was added in a quantity sufficient to moisten it and was allowed to stand for 10minutes after thorough mixing of the content. Sufficient quantity of a mixture of chloroform and ethanol (1:1) was added to soak and suspend the powder. The mixture was allowed to stand for 20minutes with occasional stirring with a rod. The mixture was filtered through a plug of cotton wool. The residue was washed twice with 2mls of chloroform and the washing was combined with the filtrate. The bulked filtrate was concentrated to dryness without overheating. The residue was allowed to cooled and dissolved in 5mls of chloroform. The chloroform solution was transferred to a small separating funnel and shaken with 3mls of dilute sulphuric acid. The two layer were allowed to separate. The chloroform lower layer was drained off and discarded. 3mls of chloroform was further added, shaken, drained off and discarded,until upper acid layer was coloured.The acid layer was made completely alkaline with strong ammonium solution ( tested with indicator paper). The extraction with the chloroform extract were refined and evaporated to dryness. The residue was dissolved in 3mls of ethanol and the test was carried out after neutralizing with dilute sulphuric acid (Brain and Turner,1975).

### Test procedure

A set of five test tubes was taken for the sample. To each amount of the ethanolic solution from the above was added dropwise, with a few drop of the following reagents; Meyer reagent ( potassium mercuric Iodine solution), Dragendroff's reagent (potassium bismuth Iodine solution), Wagner's reagent(solution of Iodine in potassium Iodide), Hager's reagent(a saturated solution of picric acid). The presence of precipitate in at least three or all of the above reagents indicates the presence of alkaloids (Evan,1989).

### Test for Terpenes and Sterols.

Five grams portion of each of the seeds powder was extracted by maceration with 50mls of 95% ethyl acetate, filtered and the filtrate was evaporated to dryness. The residue was dissolved in 10mls of anhydrous chloroform and there filtrate was divided two ( 20 equal portion and the following tests were carried out.

### Terpenes

The Lichermann-Burchard test was used to test for the presence of terpenes. The first portion of the chloroform above was mixed with 1ml of acetic anhydride followed by the addition of 1ml of concentrated sulphuric acid,down the wall of the test tube to form a layer underneath.The formation of a reddish violet color indicates the presence of terpenes (Sofowora,1993).

### Sterols

The Salkowski's test was used to test for the presence of sterols in the plant material. The second portion of the chloroform solution was mixed with 1ml of concentrated sulphuric acid carefully so that the sulphuric acid formed a lower layer. A reddish brown color indicates the presence of a steroidal ring ( Sofowora,1993).

## RESULTS

The results below revealed that *Garcinia kola* seeds extracts possess strong antibacterial activities, against the tested bacterial isolates at concentration of 50, 100, and 150mg/ml respectively. The Minimum Inhibitory Concentration (MICs) of the *Garcinia kola* extracts against the tested bacterial isolates was also determined. The MICs of the *G. kola* extracts varied between 12.5mg/ml and 25mg/ml against all the bacterial isolates used in this study.The Soxhlet Diethyl ether *G.kola* extracts (Table1),shows significant ( $P<0.01$ ) effects against all the bacterial isolates at the concentration of 50mg/ml,100mg/ml and 150mg/ml of the extracts. *Staph.aureus* had the widest zones of inhibition of 2.8,3.5 and 5.3mm. The MICs was found to be 12.5mg/ml for *Staph. aureus*, 12.5mg/ml *E.coli* and 25mg/ml for *P.aeruginosa* (Table II).

The results in (Table III),shows that Cold-water *G.kola* extracts indicates a significant ( $P<0.05$ ) activities against the bacterial isolates at various treatment regimes with *E.coli* yielding the highest zones of inhibition with 7.0mm at 150mg/ml concentration followed by *P.aeruginosa*, with 6.5mm and *Staph.aureus*, 6.0mm zones of inhibition, while at 50mg/ml, *Staph. aureus* has zone of inhibition of 4.5mm, followed by *E.coli* and *P.aeruginosa* yield 5.5mm zone of inhibition and *E.coli* has the lowest zone of inhibition of 4.5mm diameter. The MICs was found to be 12.5mg/ml for both *Staph.aureus* and *E.coli* and 25mg/ml for *P.aeruginosa* (Table IV).

The phytochemical screening of the *G.kola* extracts was conducted which revealed the presence of Flavonoids (++) , Saponin (+), Tannins (+),Sterols and Terpenes (+) and absence of alkaloids (-).

**Table 1: Antibacterial Activity of Soxhlet Diethyl ether *Garcinia kola* extracts (mm) against selected bacterial isolates.**

Concentration of extracts	<i>Staph. aureus</i>	<i>E. coli</i>	<i>Pseud. aeruginosa</i>
Treatments	(mm)	(mm)	(mm)
50mg/ml	4.5	2.8	3.5
100mg/ml	6.3	3.5	5.3
150mg/ml	7.5	5.3	6.0

**Table II: Minimum Inhibitory Concentrations of Soxhlet-Diethyl ether *Garcinia kola* extracts against the bacterial isolates.**

Mean diameter of zones of inhibition (mm) at different Concentration of extracts (mg/ml).

Test organisms	150	100	50	25	12.5	6.25	3.125	1.56
<i>Staph. aureus</i>	9.0	6.3	4.5	3.3	1.5	-	-	-
<i>Escherichia coli</i>	5.0	3.5	2.8	2.0	0.9	-	-	-
<i>Pseud. aeruginosa</i>	6.5	5.3	3.5	2.3	-	-	-	-

- = No inhibition

**Table III: Antibacterial Activity of cold water *Garcinia kola* extracts (mm) against selected bacterial isolates.**

Concentration of extracts	<i>staph. aureus</i>	<i>E. coli</i>	<i>Pseud. aeruginosa</i>
Treatments	(mm)	(mm)	(mm)
50mg/ml	4.0	3.5	3.5
100mg/ml	5.5	4.5	5.5
150mg/ml	6.0	7.0	6.5

**Table IV: Minimum Inhibitory Concentrations of Cold-Water *Garcinia kola* extracts against the bacterial isolates.**

Mean diameter of zone of inhibition (mm) at different Concentration of extracts (mg/ml).

Test organisms)	150	100	50	25	12.5	6.25	3.125	1.56
<i>Staph. aureus</i>	7.0	5.5	4.0	2.5	1.0	-	-	-
<i>Escherichia coli</i>	8.5	4.5	3.5	2.0	0.9	-	-	-
<i>Pseud. aeruginosa</i>	8.0	5.5	3.5	1.5	-	-	-	-

- = No inhibition.

**Table V: Phytochemical Screening Test.**

Test	Extracts
Flavoniods	++
Saponin	+
Tannins	+
Alkaloids	-
Sterols and Terpenes	+

**Key**

++ = present in abundance.

- = Absent (not detected).

+ = present in low concentration

## DISCUSSION

This study indicates that *Garcinia kola* seeds extract exhibits strong antibacterial activities against the tested clinical bacterial isolates at concentration of 50mg/ml, 100mg/ml and 150mg/ml. This is in

conformity with the findings, as reported by (Esimone et al, 2007), that the seeds is believed to possess many medicinal properties, which includes, anti-inflammatory, antibacterial, antimicrobial, antiurial, antidiabetic, purgative and antihepatotoxic activity (Ebana et al, 1991, Iwu, 1993, Akoechere et al, 2002, Anegbeh et al, 2006).

Moreover, Muanya (2008), have also identified bitter Kola to have strong antibiotic activities and found the plant to be very effective against disease causing microorganisms such as *E. coli*, *Staph. aureus*, *P. aeruginosa*, *Salmonella spp.*, *Streptococcus spp.*, *Candida albicans*, *vibrio cholera* and *Neisseria gonorrhoea*.

Soxhlet Diethyl ether *G. kola* seeds extracts was found to be significantly effective ( $P < 0.01$ ) against the bacterial isolates at the various treatment regimes. The mean reveal *Staph. aureus* to be the most effective bacterial that yield higher zones of inhibition at all the treatment regimes and 150mg/ml prove to be more effective as compare to 50mg/ml and 100mg/ml respectively. The MICs values for the extracts suggest the treatments at 12.5mg/ml for both *Staph. aureus* and *E. coli* and 25mg/ml for *P. Aeruginosa*. Similarly, Cold water *G. Kola* seeds extract also exhibited significant inhibitory activity against the test isolates. The treatment regimes was found to be significant ( $P < 0.05$ ), which affect the diameter yield of the bacterial isolates. The mean proves *P. aeruginosa* to be the best bacterial with the highest zones of inhibition at the various treatment regimes. This is in consistency with the investigation as reported by Ogbuhe et al (2007), on the antimicrobial efficacy of cold, hot water extracts and ethanol extract of *G. kola* which revealed that cold and hot water extract of *G. kola* moderately inhibited the growth of *Staph. aureus* and *Streptococcus pyogenes* with zone of inhibition of between 9-15mm. Also, found the cold and hot ethanol *G. kola* extracts profoundly inhibited the growth of *S. aureus*, *S. pyogenes*, *E. coli* and *S. typhi* to about 13 to 21mm. the ethanol extracts of the *G. kola*, also profoundly inhibited the growth of *S. aureus*, *S. pyogenes*, *E. coli* and *S. typhi* with zones of inhibitions ranging from 13-22mm while *vibrio spp* were not inhibited by the cold and hot extracts as well as soxhlet extracts of the plant. The phytochemical analysis of the extract of *G. kola* revealed the presence of flavonoids, tannins, saponins, sterols and Terpenes and absence alkaloids. These phytochemical compounds plays an important roles in bioactivity of medicinal plants, because the medicinal values of medicinal plant lies in these phytochemical compounds and these produce a definite and specific phytochemical action on the human body. A preliminary phytochemical test conducted by Esimone et al (2007), revealed that the most abundant phytoconstituent in *G. kola* seeds are Flavonoids, alkaloids, and tannins respectively. Other constituents are protein, Glycoside, Reducing sugar, starch, sterols and triterpenoids.

Similarly, Sofowora (1974), have also observed the presence of such constituents as flavonoids, tannins, saponins, alkaloids and glycoside,

some of which have been shown to exhibit varying antimicrobial biological activities.

Flavonoids which are part of the phytochemical constituents of *G. kola* are known to have hypoglycemic activity used in the treatments of diabetes, have been identified in this study. Anegebeh et al (2006), also reported in their finding that the antimicrobial properties of *G. kola* seeds are attributed to the flavonoids and benzophenones. Flavonoids (especially biflavonoids) have been found to be the most abundant phytoconstituent of *G. kola seeds* (Braide,1991). Flavonoids exhibit a wide range of biological activities one of which is their ability to scavenge for hydroxyl radicals and super oxide anion radicals and thus health promoting in action (Ferguson, 2001). Flavonoids also exhibit anti-inflammatory, antiangiogenic, anti-allergic effect, analgesic and antioxidant properties (Anegebeh et al, 2006, Hodek et al,2002).

Saponin has supported the useful of this plant in managing inflammation, have also been identified in this study. The chemical saponin is mainly used as tonics for the liver; it enhances the functions of the liver and gall bladder (Nwokeke, 2008). Just et al, (1998), revealed inhibiting effect of saponin inflamed cells.

Tannins, which are known for the treatment of ulcer have been identified in this study. Tannins has also been observed to have remarkable activity in cancer prevention and anticancer (Li et al, 2008). Herbs that have tannins as their component are astringent in nature and are used for the treatment of intestinal disorder such as diarrhea and dysentery (Dharmananda, 2003). Tannins exert antimicrobial activities by non deprivation, hydrogen bonding or specific interactions with vital proteins such as enzymes in microbial cells (Scalbert, 1991). Cole (1992) has identified tannins when applied to the gastric mucosa in low concentration render the outer most layer permeable and more resistant to irritation. He also indicated that tannins could also induce local vaso-constriction in small mucous. This finding supports the reasons why *G. kola* has position among medicinal plants used for the treatment of microbial infection and ailments cause by microorganisms.

Steroidal compound also present in *G. kola* seeds extract are of importance and of interest due to their relationship with such compounds as sex hormone (Okwu, 2001). The presence of steroids in plant materials suggest ones already known. It is possible that steroids occur as part of aglycone moieties of other constituents of plant like saponins and alkaloid (Harbone, 1983).

With this finding *G. kola* ranked well among the medicinal plants used routine among many tribes in Nigeria and some parts of Africa for the treatment of

infections caused by microorganisms. This also justified the facts that *G. kola* seeds is a wonder plant because almost every part of its has been found to be medicinal importance (Oguntota, 2008). It also agree with the facts that the seeds as a whole shown to have both anti-inflammatory, antiviral, antimicrobial, antibacterial, antidiabetic and antihepatotoxic activity (Iwu, 1993).

### CONCLUSION

*Garcinia kola* seeds extracts exhibited strong antibacterial activity against the tested clinical bacterial isolates at the different treatment regimes i.e. 50mg/ml, 100mg/ml and 150mg/ml concentration of the extracts. This may be attributed to the presence of these phytochemical compounds identified in this study.

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