

**INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY,
BIOLOGY AND CHEMISTRY****Research Article****Comparism of the Antimicrobial Activities of N-hexane
and Diethyl Ether Extracts of *Garcinia kola* Heckel
[*Bitter kola*] Seed****Uzundu Akueyinwa L. E¹, Utoh-Nedosa Uchechukwu A², Anowi Chinedu F³**¹Department of Pharmaceutical Technology, Faculty of Pharmacy,
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Nnamdi Azikiwe University, Awka, Anambra State, Nigeria**Abstract**

This study compared the zones of inhibition of 100mg, 50mg and 25mg concentrations of the N-hexane and diethyl ether extracts of *Garcinia kola* seeds against *Pseudomonas aeruginosa*; *Staphylococcus aureus*; *Bacillus subtilis*; *Escherichia coli*; *Klebsiella pneumonia* and *Candida albicans* using the cup or well method of assaying the extract against a Marcfarland Standard of bacterial organism seeded sterile molten nutrient agar medium maintained at 45^oC and a Sabouraud dextrose agar plate seeded with *Candida albicans* [fungus]. Diameters of zones of inhibition of the growth of the seeded organisms were determined after incubating their plates at 37^oC for 24 hours for the bacteria and at 25^oC for 72 hours for the fungus respectively [after the three aseptic 6mm holes bored in 9cm diameter solidified seeded plates had been filled with 0.25ml of (5 drops) of 100mg, 50mg and 25mg /ml dilutions of the *Garcinia kola* seed extract.. The 100mg concentrations of the diethyl- and N-hexane extracts of *Garcinia kola* seed had the highest antibacterial activity [of the 100mg, 50mg and 25mg concentrations of these extracts tested against *Pseudomonas aeruginosa*; *Bacillus subtilis* and *Klebsiella pneumonia* [with average zones of inhibition of 12.66mm, 12.66mm and 12.6mm respectively for diethyl ether and 9.66mm, 10.66mm for the N-hexane extract]. All three concentrations of the diethyl ether extract showed more antibacterial activity against *Pseudomonas aeruginosa*; *Bacillus subtilis*; and *Klebsiella pneumonia* than equivalent concentrations of N-hexane extract while at all three concentrations of the N-hexane extract showed more activity against *Staphylococcus aureus*. Both the diethyl ether and the N-hexane extracts of *Garcinia kola* showed low antifungal activity against *Candida albicans* and no antibacterial activity against *Escherichia coli*. The anti-microbial effects of diethyl ether and N-hexane extracts of *Garcinia kola* seed stem from the antispasmodic; inhibition of pathogen metabolism; hypoglycaemic; host organ-protective and anti-oxidant properties of *Garcinia kola* seed extract demonstrated in various studies and reside in the triterpenoid, flavonoid, saponin and possibly the glycoside constituents of *Garcinia kola* seed. The variation in the anti-bacterial activity of the diethyl ether and N-hexane extracts of *Garcinia kola* seed reside in the impact of the physicochemical properties of the organic solvents, diethyl ether and N-hexane on *Garcinia kola* seed extract.

Keywords: *Garcinia kola* seeds, n-hexane extract, Antimicrobial activity.

INTRODUCTION

Garcinia kola seeds are employed as a constituent of traditional medicines in West and Central Africa because of the medicinal qualities of its bitter/astringent liquid extract. In Nigeria *Garcinia kola* or bitter kola is regarded as the stronger kola [in comparison with kola acuminate because of its stronger bitter taste. In fact the Hausa ethnic group in Nigeria call bitter kola *Migin gworo* [which literally means 'husband' or 'male' kola]. These names attest to the high esteem accorded *Garcinia kola* in traditional African medicine, *Garcinia kola* plant extracts are used for the treatment of hoarseness of voice, cough, sore throat and respiratory tract inflammation; dysentery and diarrhoea; emesis; diabetes; post partum haemorrhage; cuts; parasitic skin diseases, Guinea worm infestation; liver cirrhosis and as a snake repellent, bitter tonic / astringent tonic^{3, 4, 7, 8, 9, 11} which many animal and human studies have authenticated.

A preliminary analysis of the phytochemical composition of milled dry seeds of *Garcinia kola* seeds done by us employing standard screening tests^{4, 29} showed that it contained starch, protein, glycosides, flavonoids, tannin, saponins, sterols and triterpenoids. This study compared the zones of inhibition of 100mg, 50mg and 25mg concentrations of the N-hexane and di-ethylether extracts of *Garcinia kola* seeds against *Pseudomonas aeruginosa*; *Staphylococcus aureus*; *Bacillus subtilis*; *Escherichia coli*; *Klebsiella pneumonia* and *Candida albicans* using the cup or well method of assaying the extract against microbial organisms seeded agar medium.

MATERIAL AND METHODS

Garcinia kola seeds were dried under the sun to a constant dry weight and milled with a grains-milling machine.

A portion of *Garcinia kola* seed powder was extracted by a two-step extraction process of maceration with a mixture of methanol and water (in the ratio of 9:1 in the first step and ratio of 1:1 in the second step. The two extractions were then combined and concentrated to about one third the original volume. The resultant aqueous/methanol extract was cleared of contaminants by shaking 8 times with aliquots of N-hexane (2.3 liters); concentrated again and air-dried to obtain an N-hexane soluble fraction. Another portion of *Garcinia kola* seed powder (389.6g) was defatted with petroleum ether [1.5 liters of petroleum ether]. A dark brown residue which was deposited on standing was discarded. The defatted marc (372g) was air-dried and extracted with 1-5 liters of acetone. The acetone extract was

concentrated under reduced pressure in a rotary evaporator for two hours and with an electric heater until dry (47.0g). The dry acetone extract was digested with 550mls of diethyl ether for 48 hours.

The diethyl ether soluble extract was evaporated to dryness, under low heat. 100mg/ml, 50mg/ml and 25mg/ml of the dry N-hexane soluble extract of *Garcinia kola* seed and the dry di-ethyl ether soluble extract of *Garcinia kola* seed were each dissolved in Dimethylsulfoxide [DMSO] [which also served in the study as negative control], and was each evaluated for antimicrobial activity against *Pseudomonas aeruginosa*; *Staphylococcus aureus*; *Bacillus subtilis*; *Escherichia coli*; *Klebsiella pneumonia* and *Candida albicans* by measurement of the zone of inhibition of 100mg, 50mg and 25mg concentrations of the extract to the growth of the organisms.

The determination of the zone of inhibition of the extract was done by making an overnight broth-culture of each bacterial organism to obtain a Macfarland Standard of the organism to be used to seed sterile molten nutrient agar medium maintained at 45^o C and by seeding Sabouraud dextrose agar plate with *Candida albicans* [fungus]. With the aid of a syringe, 0.25ml of (5 drops) of 100mg, 50mg and 25mg /ml dilutions of the *Garcinia kola* seed extract were filled into three 6mm holes bored in a 9cm diameter plate with an aseptic cork-borer when each micro-organism seeded agar plate had solidified. Diameters of zones of inhibition of the growth of the seeded organisms were determined after incubating the plates at 37^o C for 24 hours for the bacteria and at 25^o C for 72 hours for the fungus respectively.

RESULTS

The 100mg concentrations of the diethyl- and N-hexane extracts of *Garcinia kola* seed had the highest antibacterial activity [of the 100mg, 50mg and 25mg concentrations of these extracts tested] against *Pseudomonas aeruginosa*; *Bacillus subtilis* and *Klebsiella pneumonia* {with average zones of inhibition of 12.66mm, 12.66mm and 12.6mm respectively for diethyl ether and 9.66mm, 10.66mm for the N-hexane extract} (figure 1).

Zones of Inhibition against *Staphylococcus aureus* at both the 100mg and the 50mg concentrations were 9.00mm and 8.33mm for the 100mg and 50mg concentrations of N-hexane extract and 8.33mm and 5.33mm for 100mg and 50mg concentrations of the diethyl ether extract.

The average zones of inhibition of the 50mg concentrations of the diethyl ether and N-hexane extracts against *pseudomonas aeruginosa* and

Bacillus subtilis were 8.33mm for the 50mg concentration of both diethyl ether extract and N-hexane extract for *P. aeruginosa* and zone of inhibition of 8.66mm and 8.33mm respectively for *B. subtilis* for the diethyl ether and the N-hexane extracts.

The average zone of inhibition of the 25mg concentration of diethyl ether extract against *pseudomonas aeruginosa*, *staphylococcus aureus*; *E. coli* and *Bacillus subtilis* and *Klebsiella pneumoniae* were 2.66mm; 2.66mm; 0; 4.66mm and 6.33mm respectively for the diethyl ether extract and 3.33mm; 5.66mm; 0; 4.66mm and 2.66mm respectively for the N-hexane extract.

The average zones of inhibition of *Candida albicans* by the 100mg, 50mg and 25mg concentrations of the diethyl ether extract were 2.66mm; 1.66mm and 0.66mm respectively while those of the N-hexane Extract were 3.00mm, 1.33mm and 0.66mm respectively.

DISCUSSIONS

The 100mg concentration of the diethyl ether extract demonstrated more anti-bacterial activity than the 100mg concentration of the N-hexane extract of *Garcinia kola* Seed against *Pseudomonas aeruginosa*; *Bacillus subtilis*; and *Klebsiella pneumonia* [with a zone of inhibition of 12.66mm, 12.66mm and 12.6mm respectively against 9.66mm, 10.66mm of the N-hexane extract. However the N-hexane extract demonstrated more inhibitory activity against *Staphylococcus aureus* than the diethyl ether extract at both the 100mg and the 50mg concentrations [with zones of inhibition of 9.00mm and 8.33mm for the 100mg and 50mg concentrations respectively against 8.33mm and 5.33mm for 100mg and 50mg concentrations of the diethyl ether extract]. The 25mg of the diethyl ether extract was only fairly inhibitory against *Klebsiella pneumonia* and *Bacillus subtilis* [with zones of inhibition of 6.33mm and 4.66mm respectively] while 25mg N-hexane extract was only fairly inhibitory against *Staphylococcus aureus* and *Bacillus subtilis* [with zones of inhibition of 5.66mm and 4.66mm respectively].

The 50mg concentration of the diethyl ether and N-hexane showed about the same level of inhibitory activity towards *pseudomonas aeruginosa* and *Bacillus subtilis* [with inhibitory zones of 8.33mm for the 50mg concentration of both diethyl ether extract and N-hexane extract for *P. aeruginosa* and zone of inhibition of 8.66mm and 8.33mm respectively for *B. subtilis* for the diethyl ether and the N-hexane extracts]. The poor activity of the diethyl ether extract against *Staphylococcus aureus* was proved by the fact

that the activity of the 25mg concentration of the N-hexane extract against *Staphylococcus aureus* was slightly more than that of 50mg diethyl ether extract [with a zone of inhibition of 5.33mm for the 50mg diethyl ether extract as against a zone of inhibition of 5.66mm of the 25mg N-hexane extract of *Garcinia kola*]. The comparative antibacterial effects of the diethyl ether and N-hexane extracts are shown in figure 1.

The diethyl ether and N-hexane extracts of *Garcinia kola* seed have about the same level of anti-fungal activity against *Candida albicans* (figure 2) [with zones of inhibition of 2.66mm, 1.66mm and 0.66mm respectively for the 100mg, 50mg and 25mg concentrations of diethyl ether extract and 3.00mm, 1.33mm and 0.66mm for the 100mg, 50mg and 25mg concentrations of N-hexane extract.

The findings of this study are therefore that at all three concentrations [100mg, 50mg and 25mg concentrations] the diethyl ether extract of *Garcinia kola* seed showed more antibacterial activity against *Pseudomonas aeruginosa*; *Bacillus subtilis*; and *Klebsiella pneumonia* than N-hexane extract while at all three concentrations [100mg, 50mg and 25mg concentrations], N-hexane extract of *Garcinia kola* showed more antibacterial activity against *Staphylococcus aureus*. Both the diethyl ether extract and the N-hexane extract of *Garcinia kola* showed low antifungal activity against *Candida albicans* and both extracts showed no antibacterial activity against *Escherichia coli* (figure 2 and figure 1).

The differential sensitivity of *Pseudomonas aeruginosa*; *Bacillus subtilis*; and *Klebsiella pneumonia* on the one hand to diethyl ether extract and *Staphylococcus aureus* on the other to N-hexane extract of the same *Garcinia kola* seed is suggested to reside in the size of the molecules of the two organic solvents.

These results in which higher concentrations [100mg concentration] of diethyl ether extract demonstrated a greater anti-bacterial activity against *Pseudomonas aeruginosa*; *Bacillus subtilis* and *Klebsiella pneumonia* than N-hexane extract of *Garcinia kola* seed; the 50mg concentration of the diethyl ether and N-hexane showed about the same level of inhibitory activity against *pseudomonas aeruginosa* and *Bacillus subtilis*; the low concentration [25mg concentration] of the N-hexane extract was a little more active against *S. aureus* than the 50mg diethyl ether extract and the low concentration [25mg concentration] diethyl ether is more active against *Klebsiella pneumonia* than 50mg N-hexane extract suggest a slight difference in the content of the two organic solvent fractions. The structure of the two

organic solvents, diethyl ether and N-hexane also contribute to the differences in anti-bacterial properties of the two *Garcinia kola* seed extracts. *Klebsiella pneumoniae* was more sensitive to lighter diethyl ether extract of *Garcinia kola* seed than to the heavier N-hexane extract while *Staphylococcus aureus* was more sensitive to the heavier molecule N-hexane extract. Even the organisms *Pseudomonas aeruginosa*; *Bacillus subtilis* and *Klebsiella pneumoniae* were more responsive to the lighter 100mg diethyl ether extract than the heavier 100mg N-hexane extract. The deferential sensitivity of *Pseudomonas aeruginosa*; *Bacillus subtilis* and *Klebsiella pneumoniae* on the one hand to diethyl ether extract and *Staphylococcus aureus* on the other to N-hexane extract of the same *Garcinia kola* seed is suggested to reside in the size of the molecules and other organic solvent properties of the two organic solvents.

The antibacterial and antifungal effects shown in this study by the diethyl ether and N-hexane extracts of *Garcinia kola* seed have also been found in the stem bark and leaves² and in other studies¹⁰. These antimicrobial effects of the di-thylether and N-hexane extracts of *Garcinia kola* seed are suggested to have occurred through the antispasmodic¹; inhibition of pathogen metabolism⁵; hypoglycaemic⁸; host organ-protective^{3, 4, 7} and anti-oxidant¹¹ properties of *Garcinia kola* seed extract. These antimicrobial effects of the diethyl ether and N-hexane extracts of *Garcinia kola* seed reside in the triterpenoid, flavonoid⁹, saponin and possibly glycoside⁶ constituents of *Garcinia kola* seed. The variation in the exhibition of the anti-bacterial effects of *Garcinia kola* by the diethyl ether extract and N-hexane extracts reside in the physical and chemical properties of these two organic solvents^{3, 4} [diethyl ether and N-hexane]

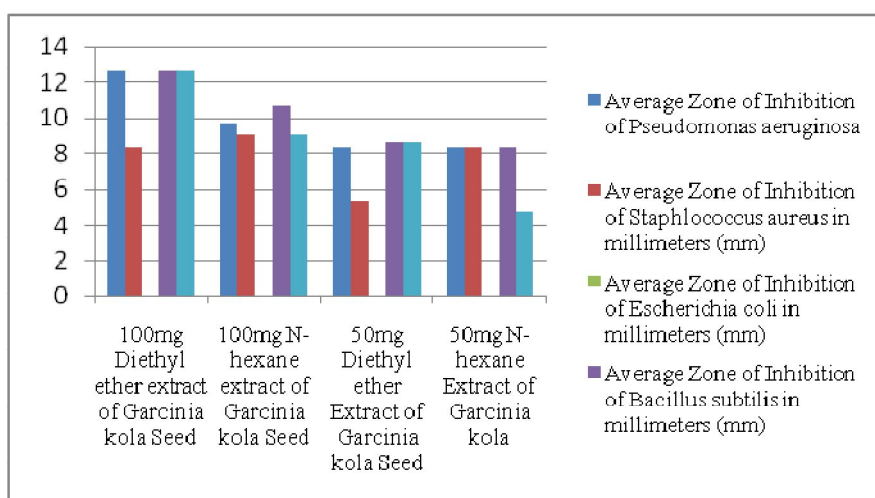


Figure 1: Comparison of the Sensitivity of Tested Pathogenic Bacteria to the 100mg and 50mg Concentrations of diethyl ether and N-hexane Extracts of *Garcinia kola* extract. The 50mg concentration of the diethyl ether and N-hexane showed about the same level of inhibitory activity towards the 5 bacterial organisms except that the diethyl ether extract showed more activity against *Klebsiella pneumoniae* while the N-hexane extract showed more activity against *Staphylococcus aureus*. The 100mg concentration of the diethyl ether extract was clearly more active than the N-hexane extract against all bacteria tested except *Staphylococcus aureus* against which N-hexane extract was a little more active than the diethyl ether extract.

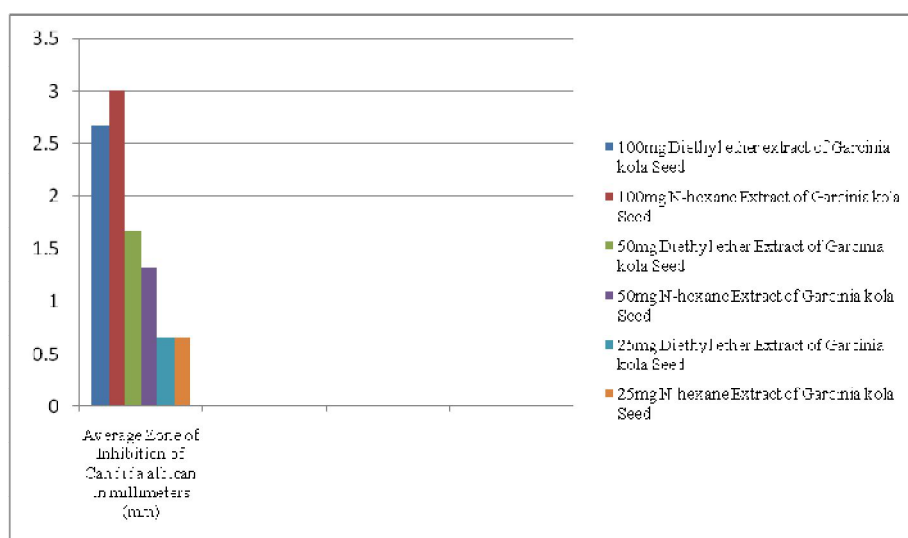


Figure 2: Comparison of the antifungal Activity of 100mg, 50mg and 25mg Concentrations of diethyl ether and N-hexane Extracts of *Garcinia kola* Seed. All three concentrations [100mg, 50mg and 25mg concentrations] of diethyl ether and N-hexane extracts of *Garcinia kola* Seed exhibited low inhibitory activity against *Candida albicans* but the 100mg concentrations of both the diethyl ether extract and the N-hexane extract showed better antifungal activity than the 50mg and 25mg concentrations [with the 100mg N-hexane extract being a little more active than the diethyl ether extract].

CONCLUSIONS

The conclusions in this study are that the diethyl ether extract of *Garcinia kola* seed showed more antibacterial activity against *Pseudomonas aeruginosa*; *Bacillus subtilis* and *Klebsiella pneumonia* than N-hexane extract the N-hexane extract of *Garcinia kola* showed more antibacterial activity against *Staphylococcus aureus*. Both extracts of *Garcinia kola* seed showed low antifungal activity against *Candida albicans* and no antibacterial activity against *Escherichia coli*.

The antibacterial and antifungal effects of the diethyl ether and N-hexane extracts of *Garcinia kola* seed have also been found in the stem bark and leaves² and in other studies¹⁰.

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