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Research Article

PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL EVALUATION OF DI-ETHYL ETHER EXTRACT OF *GARCINIA KOLA* (HECKEL) [*BITTER KOLA*] SEED

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ABSTRACT

Milled dried *Garcinia kola* seed powder was subjected to phytochemical tests to determine its constituents. Subsequently, the *Garcinia kola* seed powder was extracted using a Soxhlet extractor and the antimicrobial activity screening of the resultant di-ethylether extract of *Garcinia kola* seeds was evaluated against *Pseudomonas aeruginosa; Stappylococus aureus; Bacillus subtilis; Esherichia coli; Klebsiella pneumonia* and *Candida albicans* by determining the measurement of zones of inhibition of growth of each organism by the extract prepared in Dimethylsulfoxide [DMSO]. In-vitro assay of the extract using the cup method [three 6mm holes bored in solidified Marcfarland Standard bacteria- seeded and Sabouraud dextrose agar plate *Candida albicans*-seeded 9cm diameter agar plates with an aseptic cork-borer into which 0.25ml of (5 drops) of 100mg, 50mg and 25mg /ml dilutions of the extract were filled], was used to determine the sensitivity of the seeded organisms to the extract. Diameters of zones of inhibition of the growth of the seeded organisms were determined after incubating the plates at 37 ^{0}C for 24 hours for the bacteria and at 25 ^{0}C for 72 hours for the fungus respectively. Phytochemical screening of the *Garcinia kola* seed powder showed that components of the *Garcinia kola* seeds used in the study include starch, protein, glycosides, flavonoids, tannin, saponins, sterols and triterpenoids. *Pseudomonas aeruginosa; Bacillus subtilis;* and *Klebsiella pneumonia* were the most sensitive organisms to 100 and 50mg of the *Garcina kola* extract [to the same degree] while *Klebsiella pneumonia* was the most sensitive organism to the 25mg concentration of the extract. *Staphylococcus aureus* was the least sensitive to each of the 3 concentrations of the extract. The strongly anti-bacterial and weakly anti-fungal [inhibitory] actions of the di-ethylether extract of *Garcinia kola* seed obtained in this study are suggested to be due to the anti-oxidant and inhibitory activities of the triterpenoid (and probably also glycoside) components of the extract.

INTRODUCTION

Extraction of plant material may be done by repeated maceration with agitation; by percolation or by continuous extraction. In this study, milled dried *Garcinia kola* seeds were extracted by the Soxhlet extraction method and the antimicrobial activity of the soxhlet-extracted diethyl ether extract of *Garcinia kola* seed against *Pseudomonas aeruginosa; Stappylococus aureus; Bacillus subtilis; Esherichia coli; Klebsiella pneumonia* and *Candida albicans* was evaluated.

Phytochemical analysis of the composition of the *Garcinia kola* seed powder was also conducted.

MATERIALS AND METHODS

Garcinia kola seeds were peeled, dried under sunlight and milled into powder.

Phytochemical Screening of the *Garcinia Kola* Pulverised Coarse Seed Powder

The phytochemical analysis of the composition of the *Garcinia kola* seed powder was done by subjecting the milled *Garcinia kola* seed powder to the following phytochemical screening tests:

Test for Starch

1.5g of the *Garcinia kola* seed powder was shaken in 5ml of distilled water and two drops of iodine were added to it. The appearance of blue –black colour indicated the presence of starch.

Test for Proteins:

Two drops of Millions Reagent were added to a solution of 2.0g of the *Garcinia kola* seed Powder. The appearance of a white precipitate showed the presence of proteins.

Test for Alkaloids

2.0g of the *Garcinia kola* seed Powder was boiled with 2ml of dilute HCL in a test tube and filtered. The filterate was divided into 4 portions.

2 drops of Meyer's solution was added to portion '1'. No white precipitate was obtained which showed absence of alkaloids;

Two drops of Wagner's reagent were added to portion '2'. No brown precipitate was obtained which showed the absence of alkaloids;

Two drops of Dragendorff's solution were added to portion '3'. No orange precipitate was obtained which showed the absence of alkaloids.

Two drops of Picric Acid were added to portion '4'. No orange precipitate was obtained which confirmed the absence of alkaloids in the *Garcinia kola* seed extract.

Test for Flavonoids

2.00g of the *Garcinia kola* seed powder was dissolved in 5ml of distilled water in a test tube. Few drops of sodium hydroxide solution were added to the solution of *Garcinia kola* seed powder. A yellow colour was obtained which showed the presence of flavonoids.

10ml of ethyl-acetate was added to 0.2g of the *Garcinia kola* seed powder and heated on a water bath for 3minutes. The mixture was cooled, filtered and used for the following teats:

1. Ammonium Test

4ml of the filterate was shaken with 1ml of dilute ammonium solution.

The yellow colour obtained in the ammoniacal layer indicated the presence of flavonoids.

2. Ammonium Chloride Solution (1% Test)

Another 4ml of the filterate was shaken with 1ml of 1% ammonium chloride solution and the layers were allowed to separate. A yellow colour in the ammonium chloride layer indicated the presence of flavonoids.

Test for Tannins

1g of the *Garcinia kola Seed powder* was boiled with 5.0ml of water; filtered and used for the test.

Ferric Chloride test

Few drops of ferric chloride were added to3ml of the filtrate. A greenish black precipitate, indicated the presence of tannins (Elligitannins and gallitannins produced the blue-black colour while condensed tannins produced brownish-green-brown precipitate.

Test for Saponins

20ml of distilled water was added to 0.25g of *Garcinia kola* powder in a test tube and bolied gently in a hot water-bath for 20minutes,. The mixture was filtered hot and allowed to cool. The filterate was used for the following tests:

Frothing Test

5ml of the filterate was diluted with 20ml of distilled water and vigorously shaken and left to stand. Stable foam was observed in filterate which ndicated the precence of saponins.

Emulsion Test

2 drops of olive oil was added to the frothing solution and the contents shaken vigorously. An emulsion was formed from the frothing solution which showed the presence of saponins.

Fehling's Test

5ml of Fehling's solution [equal parts of Fehling's solution A & B] was added to 5ml of the filterate and the content was heated in a water bath. A reddish precipitate which turned brick red on further heating with added sulphuric acid indicated the presence of saponins.

Test for Glycosides

Test for Reducing Sugars

1g of the *Garcinia kola seed* powder, was boiled with 10mls of distilled water. 2mls of Fehling's solution [equal parts of Fehling's solution A & B] was added to the solution and the contents heated in a water-bath for 15 minutes. A brick red precipitate was obtained

which showed the presence of reducing sugars.

Sulphuric Acid Test for Glycosides
The brick-red precipitate was filtered off with a filter paper and the supernatant collected. 3 ml of dilute sulphuric acid was added to 5ml of the supernatant and the content heated for 15minutes; cooled and neutralized with 3ml potassium hydroxide solution [20%]. 1ml equal parts mixture of Fehlings solution A & B was added to it and the resultant solution heated for 15 minutes in a water-bath. A brick-red colouration was obtained which showed the presence of glycosides.

Ferric Chloride Test for Glycosides
3 drops of ferric chloride solution was added to another5ml of the supernatant and boiled, cooled and filtered. The filterate was shaken with equal volume of carbon tetrachloride (CCl_4) , the lower organic layer was separated. 5ml dilute ammonia was added to filterate/ carbon tetra chloride solution. A red colouration of the solution was obtained which showed the presence of glycosides.

Ethanol test and Lead acetate Test
20 ml of 50% ethanol and 10ml lead acetate solution was added to 2.og of the *Garcinia kola* seed powder and heated to in a water bath for 2 minute; cooled and filtered. The filterate was extracted twice with 15ml aliquots of. The first chloroform extract was partially evaporated in a water bath and 2ml aliquots of 3,5-dinitrobenzoic acid solution and 1ml of sodium hydroxide solution were added to it. A light violet coloration was observed which showed the presence of glycosides.
The second chloroform extract was evaporated to dryness in a water bath and dissolved in 3ml glacial acetic acid in a test tube. 2drops of ferric chloride followed by carefully added (by the side of the test tube)2ml sulfuric acid was added and the solution left to stand for 5minutes.A brownish colour which appeared at the junction of the two layers present, confirmed the presence of glycosides.

Test for Sterols and Triterpenoids
1.0g of the *Garcinia kola* seed powder was dissolved in 5ml chloroform in a test tube. This solution was poured into a saturated solution of antimony trichloride and chloroform and heated in a water bath for 10minutes.A yellow precipitate found in the chloroform layer, showed the presence of steroids and triterpenoids.

Extraction of *Garcinia kola* seed using Soxhlet Extaction Method
389.6g of the *Garcinia kola* seed powder 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.

Evaluation of the anti-microbial activity of the Diethyl Ether Extract of *Garcinia kola* Seed
Sensitivity of the following laboratory pathogenic bacteria and fungi to the diethyl ether extract of *Garcinia kola* seed was evaluated using the measurement of zones of inhibition of growth of these organisms : *Pseudomonas aeruginosa; Stappylococus aureus; Bacillus subtilis; Esherichia coli; Klebsiella pneumonia* and *Candida* albicans; by the extract as an indicator of the sensitivity of the organisms to the extract. Three concentrations of the diethyl ether extract of *Garcinia kola* [100mgl; 50mg and 25mgconcentrations], were prepared in Dimethylsulfoxide [DMSO] by preparing a stock solution and carrying out double fold dilutions on it.
An overnight broth-culture of each bacterial organism was made to obtain a Marcfarland Standard of the organism which was used to seed sterile molten nutrient agar medium maintained at 45^0 C and to seed Sabouraud dextrose agar plate with the fungus *Candida albicans*. Three 6mm holes were bored in 9cm diameter plates with an aseptic cork-borer when each micro-organism seeded agar plate had solidified.
With the aid of a syringe, 0.25ml of (5 drops) of the 100mg, 50mg and 25mg /ml dilutions of the diethyl ether *Garcinia kola* seed e were filled (in triplicate) into the three 6mm holes in the seeded agar plates. Diameters of zones of inhibition of the growth of the seeded organisms were determined after incubating the plates at 37^0 C for 24 hours for the bacteria and at 25^0 C for 72 hours for the fungus respectively.
Dimethylsulfoxide [DMSO] also served in the study as negative control.

RESULTS

The phytochemical screening tests on the aqueous extract of *Garcinia kola* seed showed that it had the following composition: starch, protein, glycosides, flavonoids, tannin, saponins, sterols and triterpenoids.

The average of 3 tests of the size of the zone of inhibition of the tested organisms showed that the 100mg and 50mg concentrations of the diethyl ether extract of Garcinia kola seed were similarly strongly active against *Pseudomonas aeruginosa; Bacillus subtilis* and *Krebsiella pneumonia* with zones of inhibition of 12.66 mm, 12.66 mm and 12.66 mm respectively for the 100mg concentration and 8.33 mm; 8.66 mm and 8.66 mm respectively for the 50 mg concentration of the extract (figure 1) . The 100mg concentration of the extract showed strong activity [zone of inhibition of 8. 33mm] while the 50mg concentration showed moderate activity [zone of inhibition of 5. 33mm] against *Staphylococcus aureus.* The 25mg concentration of the extract showed moderate activity against *Klebsiella pneumonia* and *Bacillus subtilis* [with zones of inhibition of 6.33mm and 4.66 mm respectively] and poor inhibitory activity against *Pseudomonas aeruginosa* and *Staphyloccocu aureus* [with zones of inhibition of 2.66mm and 2.66mm respectively].

All three concentrations of the diethyl ether extract of *Garcina kola* showed poor inhitory action against *Candida albicans* in the order , the activity of 100mg>50mg>25mg [with inhibition zones of 2.66 mm; 1.66mm and 0.66mm respectively] (figure 2).

All three concentrations of extract showed no inhibitory activity against *Eshcerichia coli* (figure 1).

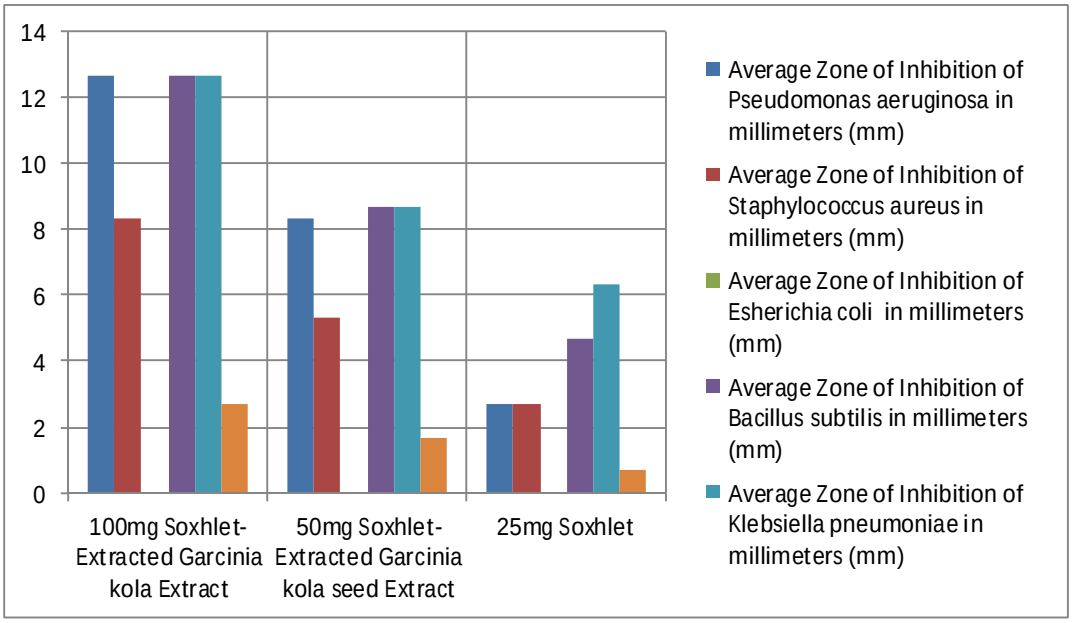

Figure 1: Average Zones of inhibition of diethyl ether extract of Garcinia kola seed against *in vitro*-assayed Bacteria. *Pseudomonas aeruginosa; Bacillus subtilis;* and *Klebsiella pneumonia* were the most sensitive organisms to 100 and 50mg of the extract [and they were sensitive to the same degree] while *Staphylococcus aureus* was the least sensitive to the extract. The lack of activity of the diethyl ether extract of Garcinia kola seed on *Esherichia coli* was shown by the white empty space in the third position of the histograms that show the activity of the 100mg, 50mg and 25mg concentrations of the extract.

Figure 2: The Low anti-fungal Activity of 100mg, 50mg and 25mg Concentrations of Diethyl Ether Extract of *Garcinia kola* seed (compare with average zones of inhibition of 12.66 mm, 12.66 mm and 12.66 mm respectively sxhibited by the 100mg concentration and average zones of inhibition of 8.33 mm; 8.66 mm and 8.66 mm respectively exhibited by the 50 mg concentration of the sane diethyl ether extract of *Garcinia kola* against *Pseudomonas aeruginosa; Bacillus subtilis* and *Krebsiella pneumonia* (figure 1) .

DISCUSSIONS

Flavonoids in *Garcinia kola* seed extracts have been shown to protect the liver from oxidative stress and organ damage. Kolaviron and *Garcinia kola* extract were shown to have hypolipidemic[2] and hypoglycaemic effects[11]. Intraperitoneally-administered kolaviron (a mixture of C-3/C-8 biflavonoids from *Garcinia kola* seed extract) , reduced the fasting blood sugar of normoglycaemic rats from 115mg/100ml to 65/100ml after 4 hours and reduced the blood sugar of alloxan-induced diabetic rats from 506mg/100ml to 285mg/ml at 12 hours[11]. Kolaviron also inhibited rat lens aldose reductase (RLAR) activity with an IC_{50} value of 5.4 x 10^{-611}. The antioxidant and organ [liver, lymphocytes etcetera]-protective activities of *Garcinia kola* seed flavonoids have been severally demonstrated in human and animal studies[1-8]. The inhibitory activity of aqueous *Garcinia kola* extract against secretion of gastric juice beyond basal normal levels was demonstrated [9, 10] [which is also an organ protective action]. Leaves and stem bark of *Garcinia kola* plant showed anti-microbial activity[4] and *Garcinia kola* extracts showed anti-fungal activity[5].Based on inference from the above cited antioxidant, anti-diabetic, anti-microbial and inhibitory activities of *Garcinia kola* extracts on gastric acid secretion we suggest that the inhibitory actions of the diethyl ether extract of *Garcinia kola* seed used in this study were due to the anti-oxidant and inhibitory activity of the triterpenoid (and probably also glycoside) components of the extract.

CONCLUSIONS

Results obtained in this study enable us to make the following conclusions:

1. The phytochemical components of the *Garcinia kola* seeds used in the study include starch, protein, glycosides, flavonoids, tannin, saponins, sterols and triterpenoids;
2. *Pseudomonas aeruginosa; Bacillus subtilis;* and *Klebsiella pneumonia* were the most sensitive organisms to 100 and 50mg of the diethyl ether extract of *Garcina kola* seed;
3. *Klebsiella pneumoniae* were the most sensitive organism while *Staphylococcus aureus* was the least sensitive organism to the diethyl ether extract of Garcinia kola seed;
4. Diethyl ether extract of Garcinia kola seed has low antifungal activity against *Candida albicans;*
5. The antimicrobial actions of the diethyl ether extract of *Garcinia kola* seed

used in this [to *Pseudomonas aeruginosa; Bacillus subtilis;* and *Klebsiella pneumonia, Klebsiella pneumonia* and *Candida albicans*] are suggested to be due to the anti-oxidant and inhibitory activities of the triterpenoid (and probably also glycoside) components of the extract.

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