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Research Article

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Phytochemical composition and antimicrobial properties of *Garcinia kola* (Bitter Kola) seed extract

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Abstract

Methaonlic and aqueous extracts of seed *Garcinia kola* were evaluated or Phytochemical properties and antimicrobial activities. Therapeutic activity of *Garcinia cola* seed extract against *Staphylococcus auerus, Escherichia coli, Pseudomonas aeruginosa*, and *Candida albicans* was studied. Results showed that Methaonlic extract of *Garcinia cola* was active against *Staphylococcus auerus, Escherichia coli* and *candida albicans*. Aqueous extract of the plant was also active against *Staphylococcus auerus* and Escherichia coli. The result from the study provides scientific evidence that *Garcinia cola* has the ability of inhibition the growth of pathogenic microorganisms. Thus, it will be helpful in the management of such infections.

Keywords: *Garcinia cola,* Phytochemical properties and antimicrobial activities.

Introduction

In our everyday existence plants is reported to important. Plants provide our food, produce the oxygen we breathe, and serve as raw materials for many industrial products such as clothes, foot wears and so many others. They also provide crude materials for our building and in the manufacture of biofuels, dyes, perfumes, pesticides and drugs. It has been shown that traditional preparations and medicinal plants with antimicrobial activities have been extensively used in the West African regions (Iwu, 1993)). It reported that bitter kola does not possess toxins. In the United States, bitter kola is termed as "Miracle drug"(Iwu, 1993)).It has been observed that Garcinia kola exhibit response to specific organs or systems in the body. *Garcinia kola* is often found in the southern part of Nigeria (Ayensu, 1978). *Garcinia kola* have antimicrobial properties, according to clinical data (Iwu, 1993).

Aim

To determine the Phytochemical composition and evaluate the antimicrobial effect of the seed extract of *Garcinia kola.*

Materials and Methods

Sample collection and processing

The seed *Garcinia kola* was obtained from kolanut dealers at free zone market Aba, Abia state. The seeds were dehusked, sun-dried four for days and ground into powder.

Source of test organisms

Pure isolates of the bacteria, *Staphylococcus auerus, Escherichia coli, Pseudomonas aeruginosa* and

a fungus, *Candida albicans* were gotten from the Department of Microbiology, Federal Medical Center Owerri, Imo state.

Culture media

Nutrient agar, macConkey agar, Sabouraud dextrose agar and lactose broth.

Extraction procedure

Methaonlic extract

20g of the ground seeds was extracted with 95% aqueous methanol (50ml) using soxhlet method. The extract was concentrated by evaporation and stored in the refrigerator until needed for test

Aqueous extract

Aqueous extract were gotten by mixing the powdered seed material of the plant (50g) with distilled water (250ml) at 90 ^{0}c in a water bath for 2 hours. They were filtered using the whattman filter paper and the filtrate was stores in a clean reagent bottle placed in a refrigerator.

Qualitative phytochemical screening of seed *Garcinia kola*

The analysis determines the biologically active compounds that are present in the seed of Garcinia kola: examples Alkaloids, saponins, tannins, flavonoids, glycosides, phlobatannin and anthraquinones.

Test for the presence of alkaloids

1g of the ground sample was boiled in 2ml of hydrochloric acid in a water bath for 5 minutes. The mixture was allowed to cool and filtered. 1ml portion of the filtrate was treated with drops of mayers reagent. A creamy white precipitate indicates the presence of alkaloids.

Test for the presence of glycosides

2g of the sample was mixed to 10ml of distilled water and heated for 5minutes in a water bath. It was filtered using Whattman's filter paper. 2mls of the filtrate was added to 0.2ml of fehling solution A and B it turns alkaline and heated in water for 5minutes. A light blue colouration was seen which indicates the presence of glycoside.

Test for the presence of tannins

0.5g if the ground sample was boiled in 20ml of distilled water in a test tube and then filtered. A few drops of 0.1% ferric acid were added and a brownish green colouration was recorded which shows the presence of tannins.

Test for the presence of phlobatannins

An aqueous extract of the plant sample was boiled with 15ml aqueous hydrochloric acid and deposition of a red precipitate was seen which shows the presence of phlobatannins.

Test for the presence of saponins

2g of the sample was boiled in 20ml of distilled water in a water bath and filtered. 10ml of the filtrate was added to 5ml of distilled water and shaken vigorously for persistent froth.

Test for the presence of flavonoids

5mls of 10% dilute ammonia solution was added to a portion of the aqueous filtrate of the plant extract, followed, by addition of concentrated H²SO4. A yellow colouration observed in the extract indicates the presence of flavonoids.

Test for the presence of anthraquinones

0.5g of the plant extract is shaken with 10ml of benzene and filtered. 5ml of 10% ammonia is mixed to the filtrate. The mixture is shaken and the presence of pink, red or violet color shows the presence of anthraquinones.

Quantitative phytochemical determination of garcinia kola seed extract

Alkaloid determination

5g of the sample was measured into 250ml beaker and 200ml of 10% acetic acid in ethanol was mixed and covered and stood for 4hours. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was mixed drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate collected and washed with dilute ammonia hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Tannin determination

0.5g of the sample was measured into 50ml distilled water and shaken for 1 hour in a mechanical shaker. This was filtered into a 50ml volumetric flask and made up to the mark. Then 5ml of the filtered was pippetted out into a test tube and mixed with 2ml of 0.1m FeCl3 in 0.1m HCl and 0.008m potassium

ferrocyanide. The absorbance was measured at 120nm within 10min.

Saponin determination

20g of ground sample was put into conical flask and 100ml of 20% aqueous ethanol was added. The sample was heated over a hot water bath for 4 hours with continues stirring at about 550^0 c. The mixture was filtered and the residue re-extracted with another 200ml 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 900^0 c. The concentrate was transferred into a 250ml separatory funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60ml of n-butanol was added to the extracts and washed twice with 10ml of 55% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the extracts were dried in the oven to a constant weight, and percentage saponin content determined.

Flavonoid determination

10g of the plant sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whattman's filter paper. The filtrate was later transferred into a crucible and evaporated to dryness over water bath and weighed to a constant weight.

Antimicrobial activity of *Garcinia kola* seed extract

Preparation and impregnation of paper nase with *Garcinia kola* seed extract

Paper discs were punched from Whattman's filter paper using an office puncher. They were used to prepare discs which were impregnated with Methaonlic and aqueous extracts of seed *Garcinia kola.* The impregnated discs were allowed to dry.

Seed dilution and zone of inhibition

10 fold serial dilutions were carried out using two loopful of the test organisms (*Staphylococcus auerus*, *Escherichia coli* and *Pseudomonas aeruginosa*). 1ml of the mixture was taken from the fourth test tube (10^3) and incubated on the prepared medium.

One disc of the Methaonlic extract was placed on each of the inoculated plate. One disc of the aqueous extract was placed on the second inoculated plate. The plates were incubated at 37 ^{0}c for 24 hours. The zone of inhibition was measured after 24 hours.

Minimum inhibitory concentration

Six test tubes were inserted in a test tube rack. The test tubes were covered with sterile cotton wool. The test tubes were labelled 10^{-1}, 10-^{2}, 10^{-3}, 10^{-4} and 10^{-5} from the second to the sixth test tube. 4ml of sterile lactose broth was added to the first test tube. 2ml of sterile lactose broth was added to the second till the sixth test tube. 1ml of the Methaonlic extract of *Garcinia kola* was added to the first test tube. Two fold serial dilution was carried out by transferring 2ml of the mixture of test tube one to test tube two (10^{-1}). The content of the test tube (10^{-1}) was mixed thoroughly and 2ml of the mixture was transferred to the third test tube (10^{-2}). This process continued to the sixth test tube (10^{-1}) and 2ml of the mixture was discarded.

0.5ml of *Staphylococcus auerus* was added to the test tubes. The same procedure was followed for *Escherichia coli* and *Pseudomonas aeruginosa.* The test tubes were covered with sterile cotton wool and were incubated at 37^C for 24 hours. The lowest concentration that shows little or no growth after 24hrs is the minimum inhibitory concentration.

Procedure for concentration

The prepared paper discs were impregnated with four different concentration of the Methaonlic extract of *Garcinia kola* (250mg/ml, 100mg/ml, 50mg/ml and 25mg/ml). The discs were air dried. The discs were placed one per plate and pressed firmly on the agar surface of *Candida albicans,* then labeled. The plates were incubated at room temperature for 24hours. Diameters of zones of inhibition were recorded in millimeters for each concentration.

Results

Table 1: Phytochemical screening of *Garcinia kola* seed extract

Bioactive components	Results
Alkaloids	+
Flavonoids	+
Glycosides	+
Phlobatannin	+
Saponin	+
Anthraquinones	+
Tannin	+

+ means Present

Table 2: Quantitative Phytochemical determination of *Garcinia kola* seed extract

Bioactive component	Result
Alkaloids	4.06
Tannin	11.00
Flavonoids	10.20
Saponins	Absent

Antimicrobial activities of garcinia kola seed extract on selected microorganisms

Table 3: Zone of inhibition of *Garcinia kola* seed extract

Diameter of zones of inhibition (mm)

Test organisms	Methaonlic extract	Aqueous extract
Staphylococcus auerus	15	10
Escherichia coli	6	5
Pseudomonas aeruginosa	Nil	Nil

Nil means no inhibition

Table 4: Minimum inhibitory concentration (MIC)

Test organisms	Tube number	MIC
Staphylococcus auerus	10^{-2}	100mg/ml
Escherichia coli	10^{-4}	250mg/ml
Pseudomonas aeruginosa	Nil	Nil

Nil means no inhibition

Table 5: Antimicrobial activity of Methaonlic extract of *Garcinia kola*

Test organisms	Concentration of extract(mg/ml)			
	250	100	50	25
	Zones of inhibition (mm)			
Candida albicans	10	8	5	Nil

Discussion

Phytochemical screening of the extract is a Swedish recorded in table **1.** The result indicates the presence of the bioactive components of interest such as alkaloids, flavonoids, glycosides, phlobatannins, saponins, anthraquinones and tannins. The bioactive components seen in this work are similar to the findings of Ezeanya *et al.* (2012) and Ukaoma *et al.* (2013) who tested for the presence of similar bioactive components in Garcinia kola seed extract.

The result of the quantitative Phytochemical determination of *Garcinia kola* seed extract is shown in table 2**.** The analysis shows a high quantity of tannin and flavonoid in the extract: Alkaloid was also present in a small quantity. No trace of saponins. The results is therefore similar to the findings of Ukaoma *et al.* (2013) who analysed quantitatively the bioactive components (alkaloids, tannin, flavonoids and saponins) of *Garcinia kola* seed extract.

The antimicrobial activities shows that *Garcinia kola* seeds extract exhibited antimicrobial effect on some of the test organisms in table 3**.** The Methaonlic extract of *Garcinia kola* showed a wider spectrum of inhibition than the aqueous extract. It was also observed that the extract was more effective in inhibiting the gram positive microorganism (*Staphylococcus auerus*). The result is similar to the first dings of Indabawa & Arzai (2011) who tested the sensitivity of *Staphylococcus auerus* and *Escherichia coli* using *Garcinia kola* seed extract. *Staphylococcus auerus* shows higher zone of inhibition compared to *Escherichia coli* and the inhibition on *Pseudomonas aeruginosa.* That is to say that *Staphylococcus auerus* and *Escherichia coli* are susceptible to *Garcinia kola* seed extract while *Pseudomonas aeruginosa* is resistant. This may be due to absence of inhibitory alkaloids against the organism. This is because alkaloids have been claimed to be responsible for antimicrobial effect (Walter and Nowaki 1978: Lehane, 1977).

The maximum dilution at which no growth occurred in the tubes was recorded as the minimum inhibitory concentration (MIC) is shown in table **4.** *Staphylococcus auerus* showed its minimum inhibitory concentration at the 2nd test tube (10^{-2}) at the concentration of 100mg/ml.

Escherichia coli was on the fourth test tube (10^{-4}) at the concentration of 25mg/ml. There was no inhibition on *Pseudomonas aeruginosa*. This result therefore agrees with that of Ezeanya *et al.* (2012) whose research was on same test organisms and extract.

Methaonlic extract of *Garcinia kola* seed showed activity against a fungus, *Candida albicans* in table **5.** Inhibition was showed at concentration ranging from 250_50 mg/ml and no inhibition at 25mg/ml. This is to show that *Candida albicans* can only be inhibited at higher concentrations of *Garcinia kola* seed extract.

Conclusion

Methaonlic and aqueous extract of *Garcinia kola* seed posseses antimicrobial activity. Garcinia kola seed extract was active against some members of enterobacteriaceae, namely - *Staphylococcus auerus* and *Escherichia coli* and a fungus - *Candida albicans.* *Garcinia kola* has a very therapeutic use as an antibacterial and antifungal agent, and for treatment of infections caused by *Staphylococcus auerus*, *Escherichia coli* and *Candida albicans*.

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