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AN APPRAISAL OF THE ANTIMICROBIAL ACTIVITY OF THE CHROMATOGRAPHIC FRACTIONS OF *GARCINIA KOLA* STEM BARK METHANOLIC EXTRACT

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ABSTRACT

The methanolic extracts of the stem bark of *Garcinia kola* was subjected to column chromatographic separation and purification. The resultant fractions were evaluated for *in vitro* antimicrobial activity against four reference bacteria (*Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa*) and two reference fungi (*Aspergilius niger* and *Candida albicans*) applying the seeded plate method. Gentamicin and ketoconazole were adopted as positive control. The results from the separation revealed five fractions which were labeled A - E. Macroscopically, each fraction exhibited a distinct colour variation such as yellow (fraction A), red (fraction B), light brown (fraction C), yellow orange (fraction D), orange (fraction E). At 25 mg/ml the zones of inhibition revealed that only fraction B had activity against *S. aureus*, while at 50 mg/ml there was activity against all the bacteria by fractions B and D. Fraction C demonstrated the least minimum inhibition. At 100 mg/ml, there was no significant increase ($p > 0.05$) in antimicrobial activity by the different fractions. No fractions had activity against the tested fungi. Nevertheless, this study evaluated what fraction of *Garcinia kola* stem bark was suitable for inhibiting the activity of susceptible organisms. Hence *Garcinia kola* stem bark can be formulated into suitable dosage forms and used for the treatment of infections by susceptible organisms.

Key words: Antimicrobial activity, *Garcinia kola*, zones inhibition

INTRODUCTION

For centuries, native indigenes of African (especially West and Central) have directly or indirectly relied on trees and herbs to provide them and their livestock with the necessary food

materials needed for metabolism and also medication for health management [1]. These trees and herbs are repositories of numerous bioactive ingredients such as flavonoids and saponins, that are useful both as sources of nutrition or as sources of therapeutic drugs in folklore medicine. Nevertheless, adequate information on the medicinal efficacy and application of these traditional tree species is on the decline and far going on extinction due to inadequate documentation of plant, the pressure of modern civilization and also consequences of uncontrolled deforestation [2].

In Africa, *Garcinia kola* Heckel (Clusiaceae) popularly perceived as "bitter kola" and nick-named as "wonder" plant, contributes immensely to ethnomedicinal development of her locality as well as medium for entertainment in traditional ceremonies. The tree is naturally located in the humid zone of tropical forests in West and Central Africa. Most parts of the plant ranging from the leaves, seed, roots and stem bark have been shown to possess some level of medicinal efficacy [3, 4].

The most adopted and utilized part of this plant is the seed or kernel, which is known for its astringent taste hence the name "bitter kola". Aside the aesthetic value of the plant, both the seed and bark of *G. kola* are used as remedies in folklore medicine for treatment of liver and gastric disorders. The seeds when masticated or chewed have the ability to suppress laryngitis, bronchitis, headaches, gonorrhea and malaria [5, 6].

Traditionally, the stem bark of *G. kola* is applied in a way similar to the seeds, to treat malaria, abdominal pains and dysentery [6]. In addition, the stem bark is used in the production of wine by enhancing both flavour and alcoholic content of traditional beverages [7, 8]. Other school of thought have demonstrated that instead of hop, *Garcinia kola* is applied in brewing lager beer which consequentially is useful in preventing beer spoilage [9]. There are countless local claims of the health benefits of *Garcinia kola* stem bark. One of such claims is that *Garcinia kola* has antimicrobial principle [10, 11]. It is on this premise that this study was objectively designed to obtain different fractions of the methanolic extract of *Garcinia kola* stem bark by chromatography separation and then investigating the different fractions obtained on the antimicrobial activity.

MATERIALS AND METHOD

Plant collection

The *Garcinia kola* stem barks were harvested from the Delta State University Botanical garden, Abraka, Nigeria. Botanical identification and authentication were performed at the Botany Department of the aforementioned Institution.

Plant extract preparation

The plant stem barks were sun dried for about three weeks and pulverized using Becker milling machine. This gave a fine powder of the plant stem and was stored at 28ºC in clean sterile bottles. All chemicals used for analysis were of analytical grade (BDH).

Collection of bacteria and fungi

The four reference bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis Pseudomonas aeruginosa*) and two reference fungi (*Aspergilius niger* and *Candida albicans*) were obtained from Biochemistry Laboratory of the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos.

Extract Preparation and Preliminary Partial Purification

Extract preparation was conducted by adopting the method of Odelola and Okorosobo [12]. Twenty-five grams of the powdered stem barks each were extracted using 200 ml of methanol and water in a Soxhlet apparatus and the extracts were dried in vacuo by a rotary evaporator. The preliminary partial purification by solvent partitioning was carried out by partitioning 1 g of the extract with solvents of the different polarities. The solvents employed were water, methanol, petroleum spirit, chloroform and ethyl acetate. This was conducted to ascertain which fraction or fractions can best exhibit antimicrobial activity via the bioactive ingredients extracted from the pulverized stem barks. The methanolic extract was mixed with 100 ml of 50% methanol and was poured in a separating funnel. It was partitioned by adding 100 ml of petroleum spirit. The aqueous and organic layers were separated. This procedure was repeated using chloroform and ethyl acetate as the organic phase [13]. The different fractions were screened for antimicrobial activity as depict in table 1.

Extract Separation and Purification

Column chromatographic technique was employed for the purification of the methanolic fraction (since this fraction exhibited activity against the organism upon preliminary partial purification). The crude mixture was introduced as narrow zone on top of the column adsorbent or support material made of silica gel. The mobile phase adopted for this particular study, consisted of methanol in varied proportions and increasing polarities to achieve optimal separations. The introduction of the mobile phase to the top of the column resulted in downward migration of the mixture of the crude and bands were collected as fractions as they emerged from the bottom of the column [14]. Extracts were reconstituted with 50% methanol in water to various concentrations for the determination of minimum inhibitory concentration (MIC). The concentrations used included 25, 50 and 100 mg/ml.

Preparation of culture and inoculation

The concentration for each fraction was screened for antimicrobial activity using the seeded nutrient agar and sabouraud carpeted plates. Culture and inoculation were prepared according to the standard procedures as described by Jahn [15], and Folkland *et al.*, [16].

For each of the bacterial seeded plates, the positive control was gentamicin at a concentration of 10mg/ml while ketoconazole was used for the fungi carpeted plates at a concentration of 20mg/ml. Procedure was replicated in triplicate.

RESULTS AND DISCUSSION

A total of 5 fractions were obtained (Plate 1) and these were labeled A to E. Macroscopic observation of the fractions showed marked variations in colour. Fraction A was yellow, while fraction B was red. Others included light brown (fraction C), yellow orange (fraction D) and orange (fraction E). The *in vitro* antimicrobial activity of the partially purified extracts using the different solvents showed that the aqueous and petroleum spirit fractions showed no activity against the organisms tested, while the methanol, ethyl acetate and chloroform fractions had activities against the Gram-positive and Gram-negative organisms but no activity against the fungi (table 1). This activity was probably due to the similar polarities of methanol, ethyl acetate and chloroform which resulted in the favourable partitioning of the active principle to the organic phase when compared to the non-polarity of petroleum spirit or extreme polarity of the aqueous extract.

The column chromatographic technique was used to separate the methanolic crude into various fractions using different combinations of solvents until 5 fractions were obtained. Table II shows the antimicrobial activity of the 5 fractions obtained when challenged against two Gram positive and two Gram negative organisms and two fungi. The positive control gentamicin and ketoconazole showed activity at the concentrations tested. The minimum inhibitory concentrations of the various fractions were determined at different concentrations. At 25 mg/ml, only fraction B had activity against *S. aureus* while there was no activity by all the other fractions. As the concentrations was increased to 50 mg/ml, there was noticeable zones of inhibition against the bacteria with the levels of inhibition in the order of fractions B > D > A> E. Fraction B had activity against *S. aureus* while fraction C had no activity against all the organisms. At 100 mg/ml of the extract, there was increase in the number of susceptible organisms. However, there was no significant increase ($p > 0.05$) in the zones of inhibition when compared to those susceptible at 50 mg/ml of extract. There was no activity against all the fungi tested at all concentrations. Despite these noticeable zones of inhibition, the values obtained were significantly lower ($p > 0.05$) than those obtained from the control (gentamicin). The major bases for the use of this plant in phytomedicines therefore stems from its relatively high safety profile which has been reported in previous studies and its ready availability in traditional medicine practice [14, 17].

Plate 1: Purification of *Garcinia kola* stem barks by column chromatography

Table I. Antimicrobial activity of partially purified extracts using the different organic solvents

Organism	AF	MF	EAF	PAF	CF
P. *aeruginosa*	-	-	9	-	9
E. *coli*	-	7	-	-	7
B. *subtilis*	-	-	7	-	-
S. *aureus*	-	9	12	-	11
A. *niger*	-	-	-	-	-

C. *albicans*	-	-	-	-	-

AF= Aqueous MF= Methanol fraction CF= Chloroform fraction EAF = Ethyl acetate fraction PAF = Petroleum spirit fraction

Table II: Zones of inhibition (mm) of test microorganism by chromatographic fractions of *Garcinia kola* stem bark at concentrations 25,50 and 100 mg/ml

Organisms	Conc (mg/ml)	A	B	C	D	E	Gentamicin	ketoconazole
P. *aeruginosa*		-	-	-	-	-	23	NT
E. Coli		-	-	-	-	-	27	NT
B. *subtilis*	25	-	10	-	-	-	26	NT
S. *aureus*		-	-	-	-	-	30	NT
A. *niger*		-	-	-	-	-	NT	21
C. *albicans*		-	-	-	-	-	NT	19
P. aeruginosa		8	8	-	9	-	26	NT
E. Coli		-	7	-	-	7	28	NT
B. subtilis	50	9	10	-	7	8	26	NT
S. aureus		-	12	-	10	-	30	NT
A. niger		-	-	-	-	-	NT	-
C. albicans		-	-	-	-	-	NT	-

P. aeruginosa		7	10	-	10	8	27	NT
E. Coli		7	10	-	8	9	30	NT
B. subtilis	100	9	11	7	9	10	32	NT
S. aureus		10	12	8	11	10	31	NT
A. niger		-	-	-	-	-	NT	-
C. albicans		-	-	-	-	-	NT	-

- No zone inhibition NT = Not Tested

CONCLUSION

This study has shown that the chromatographic fractions oil *Garcinia kola* stem barks can be used for the treatment of infections by susceptible organisms, because of its sensitivity to the extract's concentration at 50 mg/ml or more of the various extracts. The extract had activity against *S. aureus* than any other microorganism used in this study.

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