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In-vitro antimicrobial activity of methanolic and aqueous leaf extracts of *Chrysophyllum albidum* (African star apple) and *Garcinia kola* (Bitter kola)

Nicholas Chinedu Ewelike ^{1,*}, Joy Chinyere Okamadu ¹, Vincent Ezechukwu Ogwudire ² and Raymond Ikechukwu Nnadozie ³

¹ Department of Microbiology, Federal University of Technology, P.M.B 1526, Owerri, Nigeria.

² Department of Crop Science, Federal University of Technology, P.M.B 1526, Owerri, Nigeria.

³ Department of Biology, Federal University of Technology, P.M.B 1526, Owerri, Nigeria.

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Abstract

Methanolic and aqueous leaf extracts of *Chrysophyllum albidum* (African star apple) and *Garcinia kola* (bitter kola) were studied for *in-vitro* microbial activity using the disc diffusion technique. The aqueous and methanolic leaf extracts of *Chrysophyllum albidum* showed antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* but showed no antibacterial activity against *Klebsiella pneumoniae*. The methanolic leaf extract of *Garcinia kola* inhibited the growth of *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae* while the aqueous extract of the leaf inhibited the growth of *Staphylococcus aureus* and *Escherichia coli*. Both methanolic and aqueous leaf extracts of the plant showed no antifungal activity against *Candida albicans*. The minimum inhibitory concentrations of the leaf extracts of *Chrysophyllum albidum* ranged from 12.5 mgL⁻¹ to 25 mgL⁻¹ while those of *Garcinia kola* ranged from 25 mgL⁻¹ to 50 mgL⁻¹. The results obtained suggest that the leaves of these plants can be used in treating diseases caused by the test organisms. The further investigation on the crude extracts would characterize bioactive components of the leaves of *Chrysophyllum albidum* and *Garcinia kola*.

Keywords: Antimicrobial activity; Leaf extract; *Chrysophyllum albidum*; *Garcinia kola*; Minimum inhibition concentration; Zone of inhibition

1. Introduction

The use of plants by man for treatment of diseases has been in practice for a very long time. Various plant species have been exploited for their medicinal properties. Medicinal plants are plants which contain substances that could be used for therapeutic purposes or which are precursors for the synthesis of useful drugs [1]. Different parts of medicinal plants such as the leaves, bark, seeds, cotyledons and roots have been demonstrated to possess bioactive substances useful for therapeutic purpose [2,3]. Most of the plants growing in Nigeria possess medicinal properties which are utilized for curing various diseases. Herbal medicine has been widely used and formed an integral part of primary health care in China [4]. Medicinal plants have received attention of the pharmaceutical and scientific communities and various publications have documented the therapeutic values of natural compounds in a bid to validate claims of their biological activity. Attention has also been drawn on the need to carry out further investigations on the antimicrobial activity of plants and their metabolites due to challenges of growing incidence of drug resistant pathogens.

Garcinia Kola also known as bitter kola is a member of the Clusiaceae or Guttiferae family [5]. It is a tree plant that is commonly found in the tropical rain forest region of Central and West Africa. In Nigeria, *Garcinia Kola*, commonly known as bitter kola in English, 'agbilu' in Igbo (South eastern Nigeria), 'Orogbo' in Yoruba (South western Nigeria) and 'namijin

*Corresponding author: Ewelike NC; Phone: +234 806 328 9845; E-mail: cewelyke@gmail.com
Department of Microbiology, Federal University of Technology, P.M.B 1526, Owerri, Nigeria.

goro' in Hausa (North eastern Nigeria) is considered as an effective agricultural produce in the treatment of cough, diarrhoea, tuberculosis and other bacterial infections [6].

Chrysophyllum albidum is a tropical plant commonly found in the Central, Eastern and Western Africa. It is called African star apple and belongs to the Sapotaceae family. In Nigeria, the plant is commonly called 'Udara' in Igbo (South eastern Nigeria), 'agbalumo' in Yoruba (South western Nigeria) and 'agbaluba' in Hausa (North eastern Nigeria). *Chrysophyllum albidum* is used in West Africa for the treatment of different diseases [7]. The fleshy fruit pulp of the plant is eaten as snack. The fruit has been found to have high ascorbic acid [8] as well as vitamins and irons [9,10]. The seed cotyledon has been reported to possess antihyperglycemic and hypolipidemic effects [11].

In view of the nutritional and medicinal properties of the reported parts of *Garcinia Kola* and *Chrysophyllum albidum*, this research work aims at investigating the antimicrobial activity of aqueous and methanolic leaf extracts of the plants.

2. Material and methods

2.1. Collection of plant materials

Fresh leaves of *Chrysophyllum albidum* and *Garcinia kola* were collected from a rural community in Owerri West Local Government Area of Imo State, Nigeria and identified by the Department of Crop Science, Federal University of Technology, Owerri, Nigeria. The leaves were washed thoroughly with running water, rinsed with distilled water and dried with an oven at 80°C for 48 hours. Thereafter, the dried leaves were ground into fine powder using the dried leaf grinding machine (Herb Grinder).

2.2. Preparation of extracts

The leaf powder (50 g) was weighed into two different conical flasks and equal volume (200 mL) methanol and water were added to the first and second flasks respectively. The conical flasks were covered with foils to avoid solvent evaporation. The extracts were placed in a shaking incubator at 250 rpm for few minutes to ensure a uniform mixture. The mixture was left to stand at room temperature for five days. The extracts were then filtered separately using whatman's filter paper into two different sterile conical flasks. The filtered extract was centrifuged at 8000 g for 20 minutes. The supernatant was collected in sterile flask and dried. Then, it was stored at 4°C. Different concentrations of the leaf extracts consisting of 100 mg/mL, 50 mg/mL, 25 mg/mL for both aqueous and methanolic extracts of *Garcinia Kola* were used to prepare the sensitivity discs. The concentrations of the methanolic and aqueous leaf extracts of *Chrysophyllum albidum* used to prepare the sensitive discs were 100 mg/mL, 25 mg/mL and 12.5 mg/mL.

2.3. Isolation and maintenance of test organisms

The test organisms, *Staphylococcus aureus*, *Candida albicans*, *Escherichia coli* and *Klebsiella pneumoniae* used in this study were clinical isolates from the Federal Medical Centre Owerri, Nigeria. The organisms were re-identified and sub-cultured on appropriate media. The bacteria were maintained on nutrient broth at 37°C and *Candida albicans* was maintained on Sabouraud dextrose agar at 28°C.

2.4. Preparation of the test organisms

The pure isolates of the test organisms were sub-cultured on nutrient agar (for bacteria) and sabouraud dextrose agar (for *candida albicans*) media and the bacterial colonies were suspended in 10 mL nutrient broth. The bacterial cultures were incubated at 37°C for 24 hours.

2.5. Antibacterial screening

The antibacterial activity of methanolic and aqueous leaf extracts of *Chrysophyllum albidum* and *Garcinia kola* were tested by disc diffusion method. The test organisms were inoculated into sterile nutrient agar using pour plate method. The growth medium was then allowed to solidify. The prepared antimicrobial discs were aseptically picked using sterile forceps and placed at the centre of petri plates. The same was done using antibiotic discs consisting of ofloxacin and ceftriaxole as positive control and blank discs impregnated with water and methanol as negative controls. The plates were incubated at 37°C for 24 hours. The diameters of the inhibition zones were measured in millimetre (mm).

2.6. Antifungal screening

The antifungal activity of the extracts was tested by disc diffusion method. The sabouraud dextrose agar plates were inoculated with the fungal culture by pour plate method. The prepared antimicrobial discs were aseptically picked

using sterile forceps and placed at the centre of the solidified growth medium in petri plates. Black discs impregnated with solvent water and methanol were used as positive control. Nystatin was used as positive control. The activity was determined after 72 hours of incubation at 28°C. The diameters of the inhibition zones were measured in millimetre (mm).

3. Results and Discussion

The antimicrobial activity of methanolic and aqueous leaf extracts of *Garcinia kola* against the clinical isolates are shown in table 1. The results revealed that at 25 mg/mL, the methanolic extract of the leaf exhibited antibacterial activity against *Escherichia coli* (16 mm) and *Staphylococcus aureus* (12 mm). At 50 mg/mL, the leaf extract also showed antimicrobial activity against *E. coli* (21mm), *Staphylococcus aureus* (15 mm) and *Klebsiella pneumoniae* (13 mm). The zones of inhibition obtained with 100 mg/mL of the leaf extract were 24 mm, 18 mm and 16 mm against *E. coli*, *S. aureus* and *Klebsiella pneumoniae* respectively. The aqueous leaf extract of *Garcinia kola* at 25mg/L inhibited the growth of *E. coli* (15 mm) and *Staphylococcus aureus* (13 mm). At 50 mg/mL, aqueous extract also inhibited *E. coli* (19 mm) and *S. aureus* (18 mm). The zone of inhibition obtained with 100 mg/mL of the aqueous extract against *E. coli* and *Staphylococcus aureus* was 23 mm for each of the isolates. The aqueous extract of the leaf did not inhibit the growth of *Klebsiella pneumonia* at any of the concentrations.

Table 1 Results for *Garcinia kola* methanolic and aqueous leaf extracts showing measurement for zone of inhibition against test organisms

Microbial Strain	Methanolic extract			Aqueous extract		
	25 mg/mL	50 mg/mL	100 mg/mL	25 mg/ml	50 mg/mL	100 mg/mL
<i>E. coli</i>	16 mm	21 mm	24 mm	15 mm	19 mm	23 mm
<i>S. aureus</i>	12 mm	15 mm	18 mm	13 mm	18 mm	23 mm
<i>K. pneumoniae</i>	-	13 mm	16 mm	-	-	-
<i>C. albicans</i>	-	-	-	-	-	-

Table 2 Results for *Chrysophyllum albidum* methanolic and aqueous leaf extracts showing measurement for zone of inhibition against test organisms.

Microbial Strain	Methanolic extract			Aqueous extract		
	100 mg/mL	25 mg/mL	12.5 mg/mL	100 mg/mL	25 mg/mL	12.5 mg/mL
<i>E. coli</i>	19 mm	11 mm	10 mm	14 mm	10 mm	9 mm
<i>S. aureus</i>	21 mm	15 mm	13 mm	19 mm	17 mm	15 mm
<i>k. pneumoniae</i>	-	-	-	-	-	-
<i>C. albicans</i>	18 mm	14 mm	10 mm	15 mm	14 mm	12 mm

The leaf extracts of *Chrysophyllum albidum* exhibited antimicrobial and antifungal activity against the isolates (table 2). The methanolic leaf extract of the plant inhibited the growth of *E. coli* (19 mm), *S. aureus* (21 mm) and *Candida albicans* (18 mm) at a concentration of 100 mg/mL. The zones of inhibition obtained with 25 mg/mL of the methanolic leaf extract were 11 mm, 15 mm and 14 mm against *E. coli*, *S. aureus* and *Candida albicans* respectively. At a concentration of 12.5 mg/mL, the methanolic leaf extracts of the plant also inhibited the growth of *E. coli* (10 mm), *S. aureus* (13 mm) and *Candida albicans* (10 mm). The zones of inhibition obtained with the aqueous extracts of the leaf ranged from 12 mm to 19 mm. The extracts of *Chrysophyllum albidum* and *Garcinia kola* showed no antimicrobial activity against *Klebsiella pneumoniae* and *Candida albicans* respectively. Minimum inhibitory concentrations of the leaf extracts of *Chrysophyllum albidum* ranged from 12.5 mg/mL to 25 mg/mL while those of *Garcinia kola* leaf extracts ranged from 25 mg/mL to 50 mg/mL (table 3).

Table 3 Minimum inhibitory concentrations of the extracts against test organisms.

Microbial Strain	<i>Chrysophyllum albidum</i> leaf		<i>Garcinia kola</i> leaf	
	Aqueous extract (mg/mL)	Methanolic (mg/mL)	Aqueous extract (mg/mL)	Methanolic (mg/mL)
<i>E. coli</i>	25	12.5	25	25
<i>S. aureus</i>	25	12.5	25	25
<i>K. pneumoniae</i>	-	-	-	50
<i>C. albicans</i>	25	12.5	-	-

In vitro antimicrobial activity assay has been described as the first step towards the development of new chemotherapeutic agents from plants [12]. Researchers have reported the antimicrobial and anti-inflammatory properties of various plants [13,14,15,16,17,18]. These observations have helped in identifying the active agents responsible for their activities. In this study, methanolic and aqueous leaf extracts of *Chrysophyllum albidum* and *Garcinia kola* showed activity against *E. coli*, *S. aureus* and *C. albicans*. Apart from its leaf, the bioactive properties of *Garcinia kola* seed extract has also been reported [19]. Again, the aqueous fruit extract of *Chrysophyllum albidum* have been reported to possess potential antibacterial activity against some clinical isolates [20]. Similar findings have also been documented in scientific literature [21].

The antimicrobial properties exhibited by the extracts could be attributed to the bioactive substances present in these leaves. Previous researchers have characterized the physicochemical properties of *Chrysophyllum albidum* and *Garcinia kola* [22]. The antimicrobial activities of alkaloids, tannins, saponins, flavonoids, phenol and cardiac glycoside present in *Chrysophyllum albidum* and *Garcinia kola* have been demonstrated and are well established in scientific literature [23,24].

4. Conclusion

The results of this study revealed that methanolic and aqueous leaf extracts of *Chrysophyllum albidum* and *Garcinia kola* possess potential antimicrobial activity against some clinical isolates. This study discovers the possibility of using the leaf extracts of these plants in treating diseases caused by these organisms. This study will help the researchers to uncover the phytochemical properties of these leaves that are responsible for their activity against these isolates. Thus, further investigation on the crude extracts that will characterize bioactive components of the leaves of *Chrysophyllum albidum* and *Garcinia kola* may be carried out.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that no competing interest exist.

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