Original article

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF AQUEOUS AND ETHANOL EXTRACTS OF PHYLLANTHUS NIRURI AND GARCINIA KOLA

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ABSTRACT

Plants with medicinal properties popularly refers to as gift of "mother nature" to mankind are in use for centuries in the traditional system of medicines. In this study, the antibacterial and antifungal activities of different concentrations (20mg/ml, 40mg/ml, and 60mg/ml) of aqueous and ethanol extracts of *Phyllanthus niruri* (whole plant) and *Garcinia kola* seeds were determined, using agar well diffusion method, against some selected pathogenic micro-organisms. Antimicrobial susceptibility test showed the following zones of inhibition at various concentrations of the extracts respectively: Aqueous extract of *P. niruri*; *S. typhi* (18, 22, 24mm), *P. aeruginosa* (9, 11, 15mm), *E. coli* (23, 26, 30.50mm), ethanolic extract of *P. niruri*; *S. typhi* (25, 31.50, 35mm), *P. aeruginosa* (15, 19.50, 24mm), *E. coli* (27.50, 33, 38mm), aqueous extract of *G. kola*; *S. typhi* (11, 15.50, 19mm), *P. aeruginosa* (7, 11, 14mm), *E. coli* (13.50, 18.50, 22mm), ethanolic extract of *G. kola*; *S. typhi* (18, 22.50, 28mm), *P. aeruginosa* (14.50, 16, 22mm), *E. coli* (25, 29, 35mm). The results when compared to the standard controls, showed that both extracts of *P. niruri* and *G. kola* were effective against the bacterial isolates only. The minimum inhibitory concentrations (MIC) of the extracts from both samples against the test organisms, showed *E. coli* to be the most sensitive at the lowest concentration of 12.8 µg/mL, and all the test organisms, at maximum concentration of 64 µg/mL of the ethanol extract of *G. kola*. The antibacterial effects observed may be attributed to the presence of secondary metabolites in the extracts, while the non-antifungal effect could result due to resistance of the fungal strains to the active constituents of the extracts. Therefore, the extracts hold promising potential as antibacterial agents if well exploited.

Keywords: Antimicrobial, Antifungal, *Garcinia kola, Phyllanthus niruri,* Aqueous and ethanol extracts

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INTRODUCTION

Medicinal herbs have been acknowledged as the foremost means of treatment obtainable to man, and are broadly utilized by the customary medical practitioners for curing countless disease in their daily practice. Here in Nigeria, virtually all plants are medically beneficial and their use as therapeutic plants particularly in traditional medicine is presently well established and recognized as a worthwhile profession [1]. Consequent on the current existence of resistance species of almost all micro-organisms, together with side effects of most orthodox medications, concern in the use of plant and their products in the management of various health challenges is rapidly on the increase. Undeniably, nature has remained an authentic source of therapeutic means since prehistoric periods [2]. Even though the *in vivo* and *in vitro* anti-microbial properties of some these medicinal plants have been widely worked on and reported [3] [4] [5] [6], a lot of them have not actually had any scientific backing.

Herbal medicine otherwise called herbal drugs are generally of natural plants part origin, such as stems, leaves, roots, flowers, stem bark, seeds, bulb [7]. Plants contain secondary metabolites that have been linked to most of their therapeutic values [8] [9]. Phytomedical agents obtained from plants have displayed great assurance in the treatment/management of obstinate communicable diseases [9]. Bioactive constituents of plants which are of utmost importance are alkaloids, flavonoids phenolic compounds, and tannins [11], others include saponins, anthraquinones, glycosides, volatile oils, terpenes, essential oils, and carbohydrates.

The anti-bacterial activities of aqueous and methanol extracts of some therapeutic herbs reported [12] with respect to some human pathogenic microorganism, showed that the methanol extracts had widespread range of activity on these pathogenic organisms than seen in the aqueous extracts, which suggests that the methanol extracts of these selected plants may contain certain bioactive components. It was stated that methanol extract of *Aegle marmelos* and *Cassia auriculata* exhibited higher anti-bacterial activity to a group of pathogenic microorganisms [13]. The ascription of triterpenes and saponins in plants to be responsible for their analgesic, antibacterial, anti-helminthic, anti-inflammatory, anti-microbial, anti-tumor, anti-tussive activities, anti-viral, cytotoxic, fungicidal, expectorant and immuno-stimulant have been identified years ago [14].

P. niruri (Euphorbiaceae) is an annual plant commonly called stonebreaker or seed-under-leaf. It grows as 30-60 cm high, quite glabrous with its stem often branched at the base and common in rainy season. It grows as a weed, in gardens, in farms and waste-lands [15]. The name Phyllanthus means “leaf and flower” for the reason that the flower, as well as the fruit, appear to become one with the leaf. It contains phyllanthin and other constituents [16] which are known to exhibit hepato-protective activity [15]. Farmers consider it as a difficult weed but it is a treasured medicinal plant for traditional medicine practitioner. Its fruits, leaves, milky juice, roots, and whole plants are used as medicine. Fresh milky juice and powder from dried

plant are used most often in herbal preparations. It is used as leaf and seed decoction, milky latex for external application, young leaves for fevers, fresh roots against hepatitis, seeds against leucorrhoea, irregular menses, malaria, against burning micturition and boils. It has been wonderful for early stages of jaundice, indigestion, dysentery, gonorrhea, diabetes, sores, kidney stones, vaginitis, tumors, diuretics, dyspepsia, influenza, fevers and also as antiviral and antibacterial [17].

G. kola, a native medicinal tree belonging to the family *Guttiferae*. Morphologically, "bitter kola" bear a resemblance to *Allanblackia floribunda*. It is well branched, perennial and grown up to 12 m high as an average size tree in 12 years, and found in humid forests throughout Central and West Africa. It is usually called "wonder plant" due to the fact that practically every part of the plant has been seen to be of medicinal significance. It is commonly called bitter kola in English, "Agbilu" in Igbo land, "Namijin goro" in Hausa and "Orogbo" in Yoruba land of Nigeria [18]. It yields a distinctive orange-like pod with seeds covered within. The edible seed is valued in most households in Nigeria as a substitute for the true kola nuts (*Cola nitida*). By and large, the major beneficial effects of chewing this nut are seen in its mechanical cleansing and anti-microbial effect its exhibits [19].

MATERIALS AND METHODS

Plant Collection

Healthy leaves, stems, seeds of *P. niruri* (whole plant) were collected from Bosso district, Minna Niger state. The seeds of *G. kola* were obtained from "bitter kola" dealers at the railway market, Minna, Niger state. Identification of *P. niruri* and *G. kola* were carried out in the department of Plant Biology, School of Life Sciences, Federal University of Technology, Minna.

Processing of Plant Materials

The plant materials like the leaves, stems and seeds of *P niruri* were washed thoroughly with tap water for the removal of sand particles while the seeds of *Garcinia kola* were dehusked. Both the plant and seeds were then air-dried for the period of two weeks after which they were separately grounded into fine particles using mortar and pestle.

Extraction Methods

Ethanol extraction method

Air-dried and grounded plant of *Phyllanthus niruri* and *Garcinia kola* seeds 100g each, were extracted individually in 500mL 99% ethanol in the ratio of 1:5 (w/v) at room temperature for 2 days. Each of the samples were filtered with musilin cloth and Whatman No. 1 filter paper. The filtrates were then evaporated to dryness using a water bath at 70°C.

Aqueous extraction method

Air-dried and grounded plant of *P. niruri* and *G. kola* seeds 100g each, were extracted individually with 500mL distilled water at room temperature for 1 day. Each of the samples were filtered with musilin cloth and Whatman No. 1 filter paper and then evaporated to dryness using a water bath at 100°C.

Phytochemical Screening of Plant Extracts

Phytochemical screening to detect the presence of alkaloids, flavonoids, saponins, cardiac glycosides, steroids, terpenes, tannins, phenols, reducing sugars, and anthranoids, were carried out on the plant extracts using the methods of Trease and Evans (1989),

Sofowora (1993), and Harborne (1973) [20, 21, 22].

Antimicrobial Screening of Plant Extracts

Test organisms:

Pure cultures of bacteria and fungi isolates (*Salmonella typhimurium, Pseudomonas aeruginosa, E. coli, Aspergillus flavus, Aspergillus niger, and Candida albicans)* were obtained from the Department of Microbiology, Federal University of Technology, Minna. Niger State.

Preparation of media

Nutrient agar: 2.8g of powdered nutrient agar was dissolved in 100mL of distilled water inside a conical flask and corked. It was then sterilized at 121° C and a pressure of 1.5Nm^{-2} for 15 minutes in an autoclave. After sterilization, the medium was allowed to cool to 45°C (pouring temperature) and then 20mL was dispensed into sterile petri-dishes and flamed to remove any bubble. The molten agar in the petri-dishes was allowed to solidify before use under aseptic condition.

Sabouraud dextrose agar (SDA): 65g of powdered SDA was dissolved in a liter of distilled water. 0.5g of chloramphenicol powder was added so as to inhibit the growth of bacteria. It was then sterilized at 121°c for 15 minutes at a pressure of 15Nm^{-2}.

Nutrient broth: Nutrient broth powder (1.3g) was dissolved in 100mL of distilled water and dispensed into test tubes and corked. It was sterilized at 121°c for 15minutes at a pressure of 15Nm^{2}. The nutrient broth was allowed to cool at room temperature after sterilization and then used.

Standardization of inoculum

This was done by sub-culturing about 4-5 colonies from the pure growth of each test organism from the slant onto sterile nutrient broth and incubated at 37°c for 24 hours. The turbidity of the culture was compared to McFarland Standard.

Antimicrobial Susceptibility Test

The screening for antimicrobial activity begins by reconstitution of the extracts. This was achieved by dissolving 0.5g/mL, 1.0g/mL, and 1.5g/ mL of each of the extracts, after which 4mls of sterile distilled water were added to give concentrations of 0.1g/mL, 0.2g/mL, and 0.3g/mL respectively. Antimicrobial susceptibility test was evaluated using the agar-well diffusion method [23]. The nutrient agar and SDA plates containing each test organism plates were seeded with 0.2ml of each of the reconstituted extracts, giving a final concentrations of 20mg/ml, 40mg/ml, and 60mg/ml respectively. Zero point two milliliter of standard antibiotics (0.2g/mL) were introduced into the central well to serve as controls (Ampiclox- bacterial isolate and Ketoconazole- fungal isolates). The nutrient agar and SDA plates were incubated at 37°C for 24 hours and an ambient temperature (25°C-28°C) for 48 hours respectively. The diameters of clear zones of inhibition were measured and recorded in millimeters (mm).

Determination of Minimum Inhibitory Concentration (MIC)

Determination of MIC of the ethanol and aqueous extracts on the organisms were carried out using the tube dilution method [24]. Eight test tubes labeled 1-8 were selected for each organism. Each tube (1-8) contained 8ml of sterile nutrient broth. Tube 1 contained no extract which served as negative control. Two (2) ml of the reconstituted ethanol and aqueous extracts (0.2g/mL) were introduced each into tube 2 and mixed thoroughly. Two (2) ml of the content in tube 2 was transferred into tube 3. Tube 3 was mixed thoroughly

and 2ml of its content was transferred into tube 4. The procedure was then repeated for the remaining tubes up to tube 8, giving final concentrations of 40,000µg/mL, 8000µg/mL, 1600µg/mL, 320µg/mL, 64µg/mL, 12.8µg/mL, and 2.56µg/mL in each tube respectively. To these tubes (1-8), a loopful containing the test organisms were added. The tubes were then incubated at 37^0c for 24 hours and the presence or absence of growth was noted. The lowest dilution that showed no visible turbidity was regarded as minimum inhibitory concentration.

RESULTS

Phytochemicals Composition

P. niruri and *G. kola* contains alkaloids, saponins, cardiac glycoside, reducing sugar, steroids, terpenes, tannins, flavonoids, phenols and anthranoids (Table 1). Aqueous extract of *P. niruri* contained all the phytochemicals mentioned except anthranoids, Ethanol extract of *P. niruri* contained anthranoids, cardiac glycoside and reducing sugar. Aqueous extract of *G. kola* contains all phytochemicals mentioned except alkaloids, flavonoids and anthranoids. However, ethanol extract of *G. kola* shows the absence of alkaloids and anthranoids (Table 1).

Table 1: Phytochemical Compositions of *P. niruri* Plant *and G. kola* Seed Extracts.

CONSTITUENTS	APN	EPN	AGK	EGK
Alkaloid	+	+	-	-
Saponins	+	++	+	+
Cardiac Glycoside	+	-	+	+
Reducing Sugar	-	+	+	+
Steroids	+	+	+	+
Terpenes	++	+	+	+
Tannins	++	+	+	+
Flavonoids	+	++	-	+
Phenols	+	+	+	+

Anthranoids	-	-	-	-

KEY: + = present; ++ = strongly present; - = absent adopted from Sofowora (1993). APN =Aqueous *P*. *niruri* EPN =Ethanol *P*. *niruri,* AGK= Aqueous *G*. *kola* EGK= Ethanol *G*. *kola*

Antimicrobial Susceptibility Test

Table 2 shows the zones of inhibition (mm) of the aqueous and ethanolic extracts of *P. niruri* on some selected pathogenic microorganisms. The results showed dose dependent inhibitory effect against *S. typhi, P. aeruginosa,* and *E. coli*. The Ethanolic extract showed higher zones of inhibition (15.00 - 38.00 mm) compared with the aqueous extract (9.00-30.50 mm). Both extracts of *P. niruri* showed no activity on the fungal isolates as indicated by the zones of inhibitions (4.00 mm).

Table 2: Zones of Inhibition of the Aqueous and Ethanolic Extracts of *P. niruri* Plant on some Selected Pathogenic Micro- organisms.

Values of mean of triplicate experiment.

	Zone of inhibition (mm) at different concentrations (mg/mL)						
	Control	Aqueous extract			Ethanolic extract		
Test organisms	40	20	40	60	20	40	60
S. typhi	38	18.00	22.00	24.00	25.00	31.50	35.00
P. aeruginosa	59	9.00	11.00	15.00	15.00	19.50	24.00
E. coli	54	23.00	26.00	30.50	27.50	33.00	38.00
A. flavus	26	4.00	4.00	4.00	4.00	4.00	4.00
1. niger	26	4.00	4.00	4.00	4.00	4.00	4.00
C. albican	28	4.00	4.00	4.00	4.00	4.00	4.00

Any value >4 indicates some activities.

Table 3 shows the zones of inhibition of the aqueous and ethanolic extracts of G. Kola against some selected pathogenic microorganisms. The antibacterial effect of both extracts showed dose dependent pattern, however, the ethanol extract exhibited higher zones of inhibition (14.00-35.00 mm) compared with aqueous extract (7.00-22.00 mm). Both extracts showed no inhibitory effect on the fungal isolates.

Table 3: Zones of Inhibition of the Aqueous and Ethanolic Extracts of *G. kola* Seed on some Selected Pathogenic Micro-organisms.

	Zone of inhibition (mm) at different concentrations (mg/mL)						
	Control	Aqueous extract			Ethanolic extract		
Test organisms	40	20	40	60	20	40	60
S. typhi zone	38	11.00	15.50	1 19.00	18.00	22.50	28.00
P. aeruginosa	59	7.00	11.00	14.00	14.50	16.00	22.00
E. coli	54	13.50	18.50	22.00	25.00	29.00	35.00
A .flavus	26	4.00	4.00	4.00	4.00	4.00	4.00
A. niger	26	4.00	4.00	4.00	4.00	4.00	4.00
C. albican	28	4.00	4.00	4.00	4.00	4.00	4.00

Values of mean of triplicate experiment. Any value >4 indicates some activities.

Determination of Minimum Inhibitory Concentrations (MIC)

Table 4 shows the minimum inhibitory concentrations of the aqueous extract of *P. niruri* plant on some selected pathogenic microorganisms. The extract proved to effective against all the bacterial isolates at a minimum concentration of 2.56 µg/mL, and a maximum concentration of 1600 µg/mL. The aqueous extract of *P. niruri* showed no activity against the fungal isolates.

Table 4: Minimum Inhibitory Concentrations (MIC) of Aqueous Extract of *P. niruri* Plant on some Selected Pathogenic Micro-organisms.

				Concentration (µg/mL)				
Test organisms	**Negative control**	**40,000**	**8000**	**1600**	**320**	**64**	**12.8**	**2.56**
S.typhi	+	+	+	+	+	-	-	-
P.aeruginosa	+	+	+	-	-	-	-	-
E. coli	+	+	+	+	+	+	-	-
A. flavus	+	+	+	+	+	+	+	+
A. niger	+	+	+	+	+	+	+	+
C. albican	+	+	+	+	+	+	+	+

KEY: + = Turbidity; - = No turbidity

Table 5 shows the minimum inhibitory concentration of ethanolic extract of *P. niruri* plant against the bacterial isolates at 2.56 µg/mL, and a maximum inhibitory concentration of 64 µg/mL. The extract showed no Inhibitory effect on the fungal isolates.

Table 5: Minimum Inhibitory Concentrations of Ethanolic Extract of *P. niruri* Plant on some Selected Pathogenic Micro-organisms.

	Concentration (µg/mL)							
Test organisms	**Negative control**	**40,000**	**8000**	**1600**	**320**	**64**	**12.8**	**2.56**
S.typhi	+	+	+	+	+	-	-	-
P.aeruginosa	+	+	+	+	+	-	-	-
E. coli	+	+	+	+	+	+	-	-
A. flavus	+	+	+	+	+	+	+	+
A. niger	+	+	+	+	+	+	+	+
C. albican	+	+	+	+	+	+	+	+

KEY: + = Turbidity; - = No turbidity

Table 6 shows the minimum inhibitory concentrations of aqueous extract of G. kola seed against some selected pathogenic organisms. The result shows a minimum inhibitory concentration of 2.56 µg/mL, and a maximum inhibitory concentration of 64 µg/mL against the bacterial isolates. The extract showed no activity against the fungal isolates.

Table 6 : Minimum Inhibitory Concentrations of Aqueous Extract of *G. kola seed* on some Selected Pathogenic Micro-organisms.

				Concentration (µg/mL)				
Test organisms	**Negative control**	**40,000**	**8000**	**1600**	**320**	**64**	**12.8**	**2.56**
S.typhi	+	+	+	+	+	-	-	-
P.aeruginosa	+	+	+	+	+	-	-	-
E. coli	+	+	+	+	+	+	-	-
A. flavus	+	+	+	+	+	+	+	+
A. niger	+	+	+	+	+	+	+	+
C. albican	+	+	+	+	+	+	+	+

KEY: + = Turbidity; - = No turbidity

Table 7 inhibitory concentration of ethanolic extract of G. kola seed against some selected pathogenic microorganisms at 2.56 µg/mL for the bacterial isolates, and a maximum inhibitory concentration of 64 µg/mL. E. coli appeared to be more sensitive to the extract at a concentration of 12.8 µg/mL. The extract showed no inhibitory effect on the fungal isolates.

Table 7: Minimum Inhibitory Concentrations of Ethanolic Extracts of *G. kola* Seed on some Selected Pathogenic Microorganisms.

				Concentration (µg/mL)			
Test organisms	**Negative control**	**40,000**	**8000**	**1600**	**320**	**64**	**12.8**	**2.56**
S.typhi	+	+	+	+	+	-	-	-
P.aeruginosa	+	+	+	+	+	-	-	-
E. coli	+	+	+	+	+	-	-	-
A. flavus	+	+	+	+	+	+	+	+
A. niger	+	+	+	+	+	+	+	+
C. albican	+	+	+	+	+	+	+	+

KEY: + = Turbidity; - = No turbidity

DISCUSSION

Herbs are commonly used for the treatment of diseases in orthodox medicine and can also be purified and developed as standard drugs. A continued rise in antibiotic resistance has driven the necessity for development of novel antibiotics. In this study, the antibacterial and antifungal activities of aqueous and ethanolic extracts of *P. niruri* (whole plant) and *G. kola* (seed) were investigated against some selected pathogenic microorganisms. Phytochemical screening of the extracts, revealed the presence of secondary metabolites such as alkaloids, saponins, cardiac glycosides, reducing sugars, steroids, terpenes, tannins, flavonoids, and phenols in both *P. niruri* and *G. kola*, with higher concentration of these phytochemicals observed in *P. niruri* extracts (Table 1).

The aqueous and ethanolic extracts of *P. niruri* and *G. kola* were found to be effective on the bacterial isolates, with no inhibitory effect on the fungal isolates. Increase in the zones of inhibitions for each test organism, with increase in the concentration of both extracts was also observed (Table 2 & 3). The ethanol extracts of both plants were also found to be much more effective than aqueous extracts. This may be due to the improved solubility of the active compositions of the plants extracted with ethanol [29]. The study showed that *E. coli* exhibited the highest zone of inhibition of the plants extracts. The antibacterial effects of *P. niruri* extracts in this study, agrees with the work [25] who reported efficacy of *P. niruri* aqueous and ethanolic extracts against *E. coli* and *S. typhi* with no inhibitory effect on *C. albicans* [26]. The inhibitory effect of aqueous and ethanolic extract of *G. kola* seed in this study also agreed with the work [27] who reported that aqueous and ethanolic extract of *Garcinia kola* have inhibitory effect against *E. coli* but no effect on *Candida albicans.* However, [33] reported zero zone of inhibition for varying concentrations of methanolic extract of *G. kola* seed against *E. coli*. This striking difference can be attributed to the use of a different strain of the organism, that may have developed resistance to the active components in *G. kola* seed, responsible for the antibacterial effect observed in this study. The antibacterial effect of the plant extracts may be attributed to the presence of secondary metabolites in the extracts such as flavonoids, which exhibit anti-allergic effects, anti-inflammatory, analgesic and anti-oxidant properties [30]. The presence of high concentration of alkaloids in the aqueous extract of *Zapoteca portoricensis* [36], as well as several other plants extracts, has been shown to be responsible for their high antimicrobial effects, compared with other plants extracts low in alkaloids concentration [36]. Cardiac glycoside has been reported to be a unique cancer healing agent. The presence of Cardiac glycoside in *Garcinia kola* extract has been utilized for the treatment of cardiac diseases, along with other diseases such as cough, and chest pain in southwestern Nigeria. The presence of Tannins in the extracts may be effective for the treatment of bowel disorders such as diarrhoea and dysentery [31]. Steroids also present in extracts are of significance and interest due to their linkage with sex hormone [32].

The plants extracts of *P. niruri and G. kola* seed in this study showed no zone

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of inhibition against the fungal isolates, which also agreed with work [28], who reported no inhibitory effect on *A. niger*. Generally, antifungal drugs are relatively difficult to develop compared to antibacterial drugs, owing to the eukaryotic nature of the cells. Only a few classes of antifungal drugs, such as polyenes, azoles, echinocandins, allylamines, and flucytosines, are available to treat the myriad of fungal infections [35]. Antifungal resistance and host-related adverse reactions further limit the existing antifungal arsenal against fungal pathogens [36]. The continuous search for novel and safer antifungal agents from plant source has therefore become imperative.

The aqueous and ethanolic extracts of both plant and seed, when compared with the positive control showed significant difference. The positive controls had a higher inhibitory activity against the test micro-organisms at the concentration of 40mg/ml than the plant and seed extracts (Table 2 & 3). The minimum inhibitory concentration at 40,000 µg/mL, 8000 µg/mL, 1600 µg/mL, 320 µg/mL, 64 µg/mL, 12.8 µg/mL, and 2.56 µg/mL carried out on these plants extracts revealed the lowest concentration at which these plants extracts inhibits the growth of the microorganisms. Both extracts of *P. niruri* and *G. kola* exhibited strong antibacterial effects with *E. coli* been the most sensitive to the plant and seed extracts at 12.8 µg/mL (Table 4-7). Antifungal activity was not observed from both extracts of *P. niruri* and *G. kola* at the above concentrations, implying that all the fungal isolates are resistant to the active constituents of both plants extracts.

CONCLUSION

The aqueous and ethanol extracts of both *P. niruri* (whole plant) and *G. kola* (seed) were found to be effective against the bacterial isolates only, the fungal isolates were all resistant to the plants extracts. Therefore, the extracts hold a great promising potential as antibacterial agents if well exploited, especially with the growing trend of antibiotic resistance in micro-organisms, and the adverse effects associated with the use of antibiotics. There is also the need for extensive search for safer and effective antifungal agents, in order to combat the difficulties faced in the treatment of fungal infections.

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