

Original Article

Antimicrobial activity of *Phyllanthus amarus* on some human intestinal facultatively anaerobic flora

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ABSTRACT

Background: *Phyllanthus amarus* is an economic plant grown in West Africa that has antimicrobial properties. **Aim:** We investigated antimicrobial activity of aqueous extract of *Phyllanthus amarus* against some intestinal flora that are facultative anaerobes. **Methods:** The leaves were washed thoroughly in clean water, and rinsed in sterile distilled water, allowed to dry at room temperature for several days. It was oven dried at 45°C for about an hour until considered brittle enough to bleed. Final dilutions used were 500 mg/ml, 400 mg/ml, 300 mg/ml, 250 mg/ml and 200 mg/ml. Six intestinal organisms were isolated and identified: *K. pneumoniae*, *P. aeruginosa*, *S. aureus*, *E. coli*, *P. mirabilis* and *E. faecalis*. Both agar diffusion and broth dilution methods were used to assay antimicrobial activity against the organisms. **Results:** The result indicated that the growth of the organisms were inhibited at 50 mg/ml of aqueous extract by agar diffusion and broth dilution methods but varied at lower concentration. *Phyllanthus amarus* showed bacteriostatic action at this concentration because sub-culture yielded growth except on plate of *K. pneumoniae*. Consumption of cold or hot aqueous herbal preparations can alter microbial balance. The implication of ingestion of cold or hot aqueous herbal preparations against the normal intestinal flora was discussed. **Conclusion:** *P. amarus* possesses significant antimicrobial activity against normal intestinal flora.

Key words: *Phyllanthus amarus*, intestine, anaerobes, susceptibility, resistance

INTRODUCTION

Phyllanthus amarus is a small erect, annual monoecious glabrous herb that grows to 30-40 cm in height.^[1] It belongs to the family *Euphorbiaceae* with leaves that alternate distichous and crowded along lateral branchlets.^[1] About 150 species have been identified in tropical Africa.^[2] It

was reported to have originated from tropical America and has spread as weed throughout the tropics and subtropics.^[2]

Phyllanthus amarus is a plant with reported medicinal properties and broad spectrum of pharmacological activities including antiviral, antimicrobial, anti-plasmodial, anti-inflammatory, anticancer, antidiabetics, antioxidant and diuretics

properties among others.^[3] A number of active constituents of the plant are related to biologically active lignans, glycosides, flavonoids, ellagitannins and phenylpropanoids found in the leaf, stem and root of the plant along with common lipids, sterols and flavonols.^[4]

Among various research works previously done on the extract of this plant was antimicrobial potentiality. It was reported that the plant showed a significant antimicrobial activity against microbes, especially against gram-negative and gram positive organisms.^[3,5] It has also been reported that the extract has antimicrobial action against drug resistant pathogenic bacterial strains such as *Enterococcus faecalis*.^[4] The antimicrobial action was reported to be due to phyllanthin constituent.^[3]

The composition of human gastrointestinal microbiota differs and it is influenced by age, gender, diet, genetic composition, diseased and healthy state of individual and the use of antibiotics,^[6] however they are predominantly anaerobic organisms.^[7] The anaerobes are very difficult organisms to isolate and identify in the laboratory using cultural methods. Molecular methods such as qPCR and (DGGE) PCR were used to identify these anaerobes.^[8] Common gram negative facultatively anaerobic organisms such as *Klebsiella* species, *Enterobacter* species, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, and gram positive cocci are *Staphylococcus aureus*, *Peptococcus* species and *Enterococcus faecalis* are found in human large intestine.^[6] They play important roles in the maintenance of healthy intestinal micro-biota. Some of these organisms were reportedly susceptible to action of *Phyllanthus amarus*.^[3,5]

Phyllanthus amarus is used in the treatment of intestinal illness and other conditions with decoction of the aerial part or only the leaves by cool or boiled infusion in water.^[4] This plant is used mainly by oral ingestion and gets in contact with intestinal tract before absorption into other sites. The amount consumed depends on plant availability and individual judgement, and are often taken frequently.^[5]

Most of previous studies on antimicrobial activity of this plant were done on

pathogens from many sources. This work focused on antimicrobial activity of *Phyllanthus amarus* against normal microbiota of human intestinal origin and evaluated the level of antimicrobial activity of aqueous extract on some gram negative and gram positive facultative anaerobes of human gastrointestinal tract.

METHODOLOGY

Plant collection

The plant was collected from various sites at Kwara State University Campus, Malete and within Ilorin metropolis during the rainy season in June/July. This herb was identified and authenticated at the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. The leaves and the beads were painstakingly picked from the front and thoroughly washed in clean water, then rinsed in distilled water. It was allowed to air dried at room temperature for several days, and then oven-dried at 45°C for one hour until it was considered brittle enough for blending into fine powder. This was sieved to remove bigger particles, then re-blended and sieved again.

Extract preparation

Two hundred grams of the powder was suspended in 1,000 ml of sterile distilled water in a conical flask. The flask was placed on orbital shaker (Gem Instrument, Japan) and set at 120 rpm for 8 hours. It was boiled to 100°C, allowed to cool and then filtered using Whiteman No 1 filter paper. The preparation of the extract was carried out by hot distillation using Soxhlet extractor (Quickfit, UK). The water extract was obtained at 100°C. The extract was reduced to near dryness by freeze-drying.

Isolation and identification of intestinal organism

Six intestinal organisms from individuals without history of intestinal illness were isolated and identified at the Medical Microbiology and Parasitology Department, University of Ilorin Teaching Hospital, Ilorin, Nigeria. The organisms were *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Escherichia coli* and *Pseudomonas aeruginosa*. These organisms were sub-cultured on Blood agar and MacConkey agar (LAM M, UK) to check for organism purity, from where suspensions were made in sterile normal

saline and adjusted to 0.5 McFarland standards.^[9]

Treatment

Ten grams of the extract was weighed into 50 ml of sterile distilled water to give 200 mg/ml. This was further diluted to 50 mg/ml, 40 mg/ml, 30 mg/ml, 25 mg/ml and 20 mg/ml as determined from trial study. Sterile swab stick dipped into appropriate test organism suspended in normal saline was used to uniformly seed previously prepared and dried Mueller Hinton agar (LAM M, UK). Flame sterile cork borer of 5 mm in diameter was used to make wells of 2.5 cm apart. The wells were labelled with appropriate concentration of the aqueous extract. The various concentrations were dropped into each well to fullness using sterile Pasteur pipette. Ofloxacin antibiotic suspension at concentration 10 µg/ml and sterile distilled were used as control. Triplicate set of plates were made for each of the organisms. These plates were incubated at 37°C for 18 hours. Zones of inhibition around each well was measured in millimetre and used to determine microbial activity of the aqueous extract.^[10]

Broth dilution

Another 100 grams extract was weighed into 100 ml of sterile distilled water. It was shaken until completely dissolved. This dilution gave 1,000 mg/ml. From this stock dilution, other dilutions were made into 500 mg/ml, 400 mg/ml, 300 mg/ml, 250 mg/ml, 200 mg/ml. Nine ml of peptone water was prepared in MacCartney bottles and 1 ml of each of the dilutions was added to give a final dilution of 50 mg, 40 mg, 30 mg, 25 mg and 20 mg/ml respectively. A drop each prepared organisms was added to each set of preparations. The control was

extract-free peptone water with test organisms. The broths were incubated at 37°C for 18 hrs.^[9] Minimum inhibition concentration (MIC) is defined the highest dilution that showed no growth.^[9] All the broth cultures were also sub-cultured on Mueller Hinton agar do determine bactericidal action. The diameters zones of inhibition of the organisms were measured in triplicates with a set of divider and ruler and the mean value of each set of reading obtained. The wells in which there was no visible inhibition or inhibition that was too small to be measured were recorded as resistance. Zone diameter less than or equal to 6 mm were considered resistant.^[9] Zone diameter of 8-10 mm were considered moderately sensitive, while those greater than 10 mm are considered susceptible.^[9]

RESULTS

The antibacterial activity of the aqueous extract as determined by agar well diffusion method is as shown in table 1. In the serial dilution in peptone water, at lower dilution of 20 mg/ml, 25 mg/ml and 30 mg/ml, there were turbidity, indicating growth at these concentrations for all tested intestinal flora. At higher concentration of 40 mg/ml and 50 mg/ml, there was no turbidity except in these organisms: *K. pneumoniae*, *S. aureus* and *P. mirabilis*. Upon subculture on Mueller Hinton agar, only *P. mirabilis* showed no growth at 50 mg/ml concentration of aqueous extract.

Table 2 depicts antibacterial effect in broth dilution of aqueous extract of *Phyllanthus amarus* on intestinal flora.

Table 1: Concentrations of aqueous extract of *Phyllanthus amarus* against intestinal flora on agar diffusion test

Organism	Concentration of aqueous extract (Diameter of zone of inhibition in mm)											
	20 mg		25 mg		30 mg		40 mg		50 mg		Ofloxacin	
	Zone	Rating	Zone	Rating	Zone	Rating	Zone	Rating	Zone	Rating	Zone	Rating
<i>P. aeruginosa</i>	-	R	-	R	-	R	6mm	R	18mm	S	15mm	S
<i>S. aureus</i>	-	R	6mm	R	10mm	M	15mm	M	17mm	S	24mm	S
<i>E. faecalis</i>	-	R	-	R	-	R	6mm	R	10mm	M	14mm	M
<i>E. coli</i>	-	R	8mm	R	17mm	S	18mm	S	18mm	S	20mm	S
<i>P. mirabilis</i>	-	R	6mm	R	12mm	M	16mm	M	17mm	S	24mm	S
<i>K. pneumoniae</i>	-	R	-	R	6mm	R	6mm	R	17mm	S	18mm	S

Key: R-Resistance, M-moderately sensitive, S-sensitive

Table 2: Antibacterial effect of aqueous extract of *Phyllanthus amarus* on intestinal flora in broth dilution test

		Concentration of aqueous extract (Diameter zone of inhibition in mm)					
		20 mg	25 mg	30 mg	40 mg	50 mg	
Ofloxacin Organism							
<i>P. aeruginosa</i>	Broth	t	t	t	t	nt	nt
	Subculture	g	g	g	g	g	ng
<i>S. aureus</i>	Broth	t	t	t	nt	nt	nt
	Subculture	g	g	g	g	g	ng
<i>E. faecalis</i>	Broth	t	t	t	t	t	nt
	Subculture	g	g	g	g	g	g
<i>E. coli</i>	Broth	t	t	t	t	nt	nt
	Subculture	g	g	g	g	g	ng
<i>P. mirabilis</i>	Broth	t	t	t	nt	nt	nt
	Subculture	g	g	g	g	ng	ng
<i>K. pneumonia</i>	Broth	t	t	t	nt	nt	nt
	Subculture	g	g	g	g	g	ng

Key: t-Turbid; nt-Not Turbid; g: Growth; ng-No Growth

DISCUSSION

The use of *Phyllanthus amarus* as herbal medicine had been well reported^[11] because of its health enhancing properties that including antibacterial, antioxidants and anti-inflammatory, the plant had been referred to as 'the wonder plant'.^[12]

Unlike most of the previous research work, this present study investigated the antibacterial effect of this plant on bacteria that are normal flora of human gastrointestinal tract (microbiota). The result showed that aqueous extract of the plant leaves have antibacterial activity on normal flora of human intestinal tract. At 50 mg/ml of the plant extract, all the organisms tested showed susceptibility in broth dilution and agar diffusion methods except *E. faecalis*. This same organism showed moderate susceptibility to ofloxacin. This finding is similar to the report of Saranraj and Sirasakthivelan that reported susceptibility of eight organisms to *Phyllanthus amarus* including all the ones used in this study.^[12] This result was also in consonance with the findings of Olufemi and Debiri that reported antibacterial activity of *Phyllanthus amarus* against four multiple resistant bacteria;

although they used higher concentration of aqueous extract of 100 mg/ml and 150 mg/ml than used this present study.^[5]

In broth dilution of aqueous extract of the plant, *Proteus mirabilis*, *Klebsiella pneumoniae* and *Staphylococcus aureus* were more susceptible to the extract with no growth (no turbidity) at 40 mg/ml and 50 mg/ml but other organisms have growth (showed turbidity). When sub-cultured on Mueller Hinton agar, all but *Klebsiella pneumoniae* showed no growth at 50 mg/ml. This showed that *Phyllanthus amarus* has only bacteriostatic effect on all the organisms except on *Klebsiella pneumoniae* that the plant has bactericidal affect at 50 mg/ml.

Previous reports indicated that approximately 60-80% of the world's inhabitant still depends on natural plant products for remedies for common illnesses.^[13] Some of these herbs are taken either as cold or hot aqueous infusion with little consideration for dosage and are taken as often desired or recommended. The current study showed that whether taken for antibacterial purpose or other ailments, *Phyllanthus amarus* has inhibitory effect on the normal facultatively anaerobic bacteria found

in human gastrointestinal tract. Vrieze and colleagues showed that the gastrointestinal tract microflora plays an important role in health status of the host as it contributes to overall metabolism, physiology and plays a role in converting food into nutrients and energy.^[14,15] Most of the intestinal microbes therefore have a profound influence and crucial role for human life. Any antibiotics or herbal infusion (cold or hot) that would alter the protective role microbiota and result in increased susceptibility of mucosal to infection should be ingested with caution or under proper supervision.

In this study, only some facultative anaerobes of intestinal origin were tested. However, the study can be expanded to include the antimicrobial effect of the plant extract on the gram positive and gram negative anaerobic organisms that form predominant organisms of the colon.

In conclusion, *Phyllanthus amarus* inhibits growth of intestinal microbiota tested in this study. It should thus be consumed with caution. We recommend that herbal preparations such as *Phyllanthus amarus* that have high bioactivity should be subjected to animal and human studies to determine their effectiveness in whole-organism system, especially effects on beneficial microbiota. It would profit humanity if only standardized dosage of these beneficial herbal preparations is often administered. This would avoid unwarranted complications on humans.

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Conflict of Interest: None declared



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