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Full Length Research Article

Antimicrobial Effect of *Phyllanthus amarus* and *Parquetina nigrescens* on *Salmonella typhi*

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ABSTRACT

Phyllanthus amarus and *Paraquetina nigrescens* are economic plants grown in West Africa for antimicrobial properties. Crude aqueous (hot and cold water) and ethanolic extracts of the plants were investigated for antimicrobial activity against *Salmonella typhi*. The organism was collected from the University College Hospital, lbadan, Nigeria and was exposed to ten standard different antibiotics and also to crude extract of *P. amarus* and *P. nigrescens*. Agar cup diffusion method was employed fro the plants extracts while disk diffusion method for the standard antibiotics. Ethanolic extracts of *P. amarus* had the strongest activity against *Salmonella typhi* with 8.0mm zone of growth inbibition followed by hot water (4.7mm) and cold water (3.8mm). This was statistically significant at P= 0.05 when compared with hot and cold water extracts. Amongst the commercial antibioticis examined, ciprofloxacin had the highest zone of growth inhibition of 9.0mm; Oflxation (6.0mm) Amoxycillin, (4.0mm) while other antibiotics had on effect on test organism. Screening carried out on *P. amarus* and *P. nigrescens* using standard methods revealed the presence of saponins, alkaloids, tannins and cardiac glycosides. P. amarus possesses significant antimicrobial activity and confirms the justification by herbalists as extract used for treatment of typhoid fever.

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***Key Words*:** *P. amarus, P. nigrescens, antimicrobial activity, standard antibiotics, typhoid fever*

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INTRODUCTION

There has been an increasing interest world wide on therapeutic values of natural products. It is believed that the cure to any debilitating human ailments and diseases may be found among the world's flora in nature's pharmacy (Olowosulu and Ibrahim, 2006). In addition, nature has presented to humanity the gift of vast therapeutic workshop with a wide variety of medicinal plants. There are multitudes of potential useful bioactive substances to be derived from these plants. These phytochemicals have made significant contribution in maintaining human health. There are multitudes of potential useful bioactive substances to derive from these plants. Theses phytochemical have made significant contribution in maintaining human health. The significant of drugs derived from plants cannot be over emphasized with the recent trend of high percentage of resistance of microorganisms to the present day antibiotics (Ibekwe et al, 2000). Effort has been intensified by researcher towards a search for more source of antimicrobial agents. Phyllanthus amarus is a herb common to central and southern lndia and can grow to 30-60cm in height. Al parts of the plant are employed therapeutically. Phyllanthus species can also be found in other countries including China (e.g. P. uminaria), The Phillipines, Cuba, Nigeria and Guam (Bharatiya, 1992). It blocks DNA polymerase in the case of hepatitis B virus during reproduction. In one study, 50% of those infected with chronic viral hepatitis B lost one of the major blood markers of hepatic B virus infection (e.g. hepatitis B surface antigen) after using Phyllanthus for 30 days.

Parquetina nigrescens is commonly found in secondary forest and around villages in Senegal and Nigeria. It is a perennial plant with twining stems and a woody base, shortly tapering 10-15cm long, 6-8cm broad, smooth, long stem. The leaves and whole plant are usually used for the treatment of gonorrhoea, jaundiced, rickets and asthma (Schlage et al, 1992). It is mostly used by traditional healers (Leaman et al, 1995). This paper, therefore, reports investigation into the antibacterial activities of extracts of P. amarus and P. nigrescens against clinical isolate of S. typhi and the results of preliminary phytochemical screening of the aforementioned plants.

MATERIALS AND METHOD

Plant Collection and Identification: The two plant used in this study, Phyllanthus amarus and Parguetina nigrescens were both collected from the Department of Forestry, University of Agricultural, Abeokuta. The plants were identified by carrying out macroscopical examination on plant samples as stipulated by Dalziel (1968) and confirmed at Department of Forestry, University of Agriculture, Abeokuta, Nigeria. The leaves of both plants were chopped into small pieces and thereafter, pulverized in a domestic mill. The powdered mass was later used for extraction.

Preparation of Plant Extracts: Twenty grammes (20g) of the pulverized leaves of each plant was decocted with 100ml of cold water left overnight, hot water (100^oC) for 5 minutes), and ethanol. Prior to decoction, the leaves were soaked in the extracting solvent for 3 days. The mixture was then filtered and the filtrate evaporated to semi solid mass using a rotary evaporator (Brichi, Germany) (Olowosolu and Ibrahim, 2006) and subsequently drying in a beaker on water bath to give a dark resinous mass. The plant extracts from the various solvents were reconstituted using 10% v/v ethanol as solubilising agent at concentration of (10 and 100)mg/ml for antimicrobial activity evaluation.

Test organism: Test organism used for this study was *salmonella typhi*. The stock culture maintained on nutrient agar was obtained from the Department of Medical Microbiology, University College Hospital, Ibadan, Nigeria.

Preparation of Inoculum: A loopful of *S.typhi* was taken and sub-cultured in test tube containing 10ml of nutrient broth. The test-tube was incubated at 37^oC for 24 hours. The broth was standardized using sterile normal saline to obtain a population of 10^4cful/ml. Antimicrobial Studies. Two approaches were used for the evaluation of

antimicrobial activity of both plant extract and commercial antibiotics.

(a) Agar Cup Diffusion Method: Agar cup diffusion method described by Hugo and Russel (1996) was employed.

An overnight culture of *S.typhi* was standardized to contain approx. 10^7cfu/ml was inoculated into 20ml of molten nutrient agar. The culture medium was allowed to set. Thereafter, a sterile cork borer N. 4 (8.0mm diameter) was used to punch wells in the seeded nutrient agar. The agar plugs were removed with a flamed and cooled wire loop. Into the separate well was poured different concentrations of the various plants extracts. The plates were incubated at 37^{o}C for 24 hours and the zone of inhibition was measured. The experiments were repeated in triplicates.

(b) Disk Diffusion Method: Disk diffusion method was employed to determine the effect of standard antibiotics against the test microorganism. Standard antibiotic disc with ten different antibiotics were used against *S. typhi*. The nutrient agar plates were seeded with *S. typhi* and filter paper strips of standard antibiotics (Nitrofurantoin, Ciprofloxacin, Gentamycin, Ampicillin, Cefuroxin, Chloramphenicol, Ofloxacin, Amoxyllin, Norfloaxacin, Tetracycline) were laid aseptically on the plate using a pair of forceps. The plates were incubated at 37^{o}C for 24 hours. After incubation, zone of growth inhibition was measured and recorded.

RESULTS

Table 1 shows the zone of inhibition of *P. amarus* against *S. typhi*. At 24 hours contact period, ethanolic extract of P. amarus exerted greater inhibitory activity against *S. typhi* with a zone of 8.3mm in diameter

The zone of inhibition of ethanolic extract of P. amarus on S. typhi decreased from 8.3 mm to 7.7mm as the contact time increases from 24 – 72 hour. The zone of inhibition using cold and hot water extract of the same plants were much lower, however, the same decreasing pattern repeated itself as the contact time incersased.

Table 2 shows the zones of inhibition of Parquetina nigrescens on S. typhi. The values were much lower than those obtained with P. amarus. These value range from 3.3mm of cold water extract to 3.7mm ethanolic extracts.

As contact time with organism increased, the zones of inhibition decreased. Commercial antibiotics were compared with plant extract of P. amarus and their antimicrobial activity against S. typhi was recorded. The highest microbial activity was exhibited by Ciprofloxacins.

Table 1: Diameters of Zones of Growth Inhibition (MM) of Phyllanthus on *S. typhi.* Inhibition zone (diameters in mm) + _ SD

Contact time (Hours)	Cold water Extract (mg/ml)	Hot water Extract (mg/ml)	Ethanol extract (mg/ml)
24	4.0 ± 0.10	5.2 ± 0.14	8.3 ± 0.12
48	3.7 ± 0.08	4.5 ± 0.09	8.0 ± 0.10
72	3.2 ± 0.08	4.3 ± 0.08	0.77 ± 0.06

Table 2: Diameters of Zones of Growths Inhibition of *Parquetina nigrescens* on S. typhi

Contact time (Hours)	Cold water Extract (mg/ml)	Hot water Extract (mg/ml)	Ethanol extract (mg/ml)
24	3.3 ± 0.06	3.2 ± 0.03	3.7 ± 0.03
48	3.3 ± 0.06	3.0 ± 0.05	3.5 ± 0.00
72	3.3 ± 0.06	2.7 ± 0.03	3.5 ± 0.00

Table 3: Zones of inhibition of standard antimicrobial agents S. typhi.

Standard agents	Zone of inhibition (mm) + SD
Nitrofurantion	0.0 ± 0.00
Ciprofloxacin	9.0 ± 0.01
Gentaniycin	Nil
Ampicillin	Nil
Cefuroxin	Nil
Chloraphenicol	0.0 ± 0.01
Ofloxacin	6.0 ± 0.00
Amoxycillin	4.0
Norfloxacin	Nil
Tetracycline	Nil

Table 4: Mean separation of both standard and crude antimicrobials using Dwuncan multiple range test

Antimicrobial Plant	Zone of inhibition (mm)	Duncan multiple range
Nitrofurantion	0.0	f
Ciprofloxacin	9.0	a
Gentaniycin	0.0	f
Ampicillin	0.0	f
Cefuroxin	0.0	f
Chloraphenicol	0.0	f
Ofloxacin	6.0	c
Amoxicillin	4.0	d
Norfloxacin	0.0	f
Tetracycline	0.0	f
Phyllanthus amarus (Ethanol)	8.0	b
Parquetina nigrescens (ethanol)	3.6	e

Means within the column with the same alphabet are not significantly different

Ciprofloxacin had the highest zone of inhibition of 9.0mm against *S. typhi* followed by Ofloxacin (6.0mm), Amoxylcillin (4.0mm) while the rest antibiotics showed no effect on *S. typhi.*

Table 4 shows the means zones of inhibition of commercial antibiotics (standard) and local crude extracts of P. amarus and P. nigrescens. This results revealed that P. amarus followed closely Ciproxacin in antimicrobial activity than all other commercial antibiotics tested.

Phytochemical analysis of P. amarus and P. nigrescens were carried out. Preliminary screening revealed the presence of tannins, saponins, cardiac glycosides and alkaloids.

DISCUSSION

Several investigations had reported that plants contain antimicrobial substances (El-Said et al, 1971, Lewis, 1980; Zaria *et al* 1975, Ibekwe *et al*, 2001, Akujobi *et al*, 2004). The results of the present study agree essentially with the reports of these previous workers. The result shows that cold and hot water extracts of Phyllanthus amarus were not as effective in antimicrobial activity against S. typhi as ethanolic extracts. This is not surprisingsince ethanol is generally able to dissolve multivariable types of compounds; polar and non-polar, simple and complex chemical structures compared with chloroform which solubilizes mainly flavenols (Phenolic compounds from plant) (Cowan, 1999). The relative amount of phytochemical substances from plant extraction depends on the solubility of the phytochemical in the solvent used for extraction (Olowosulu and Ibrahim 2006).

Although ten commercial antibiotics (standard) were tested against S. typhi, only three (Ciplofloxacin Ofloxacylin and Amoxycillin) had antimicrobial effect in decreasing order. Ciprofloxacin had the highest zone of growth inhibition against S. typhi. And remarkably has become the antibiotics of choice in the treatment of typhoid fever. Unfortunately, resistance of salmonella typhi strains to all of these antibiotics is becoming more common globally. As such, appropriate treatment varies with geographic distribution of resistant strains.

The antimicrobial properties exhibited by the extracts may be associated with the presence of tannins, sapoins, cardiac glycosides and alkaloids found in the plant extracts. a large number of flavonoids have been reported to possess antimicrobial properties (Bastista *et al.,* 1994; Tsuchiya *et al* 1996; Boris, 1996; Olowusulu and Ibrahim, 2006; Akimnjobi *et al* 2006). Tsuchiya *et al* (1996) attributed the antimicrobial activities of flavonoids to their ability to complex with extracellular and soluble proteins as well as their ability to complex with bacterial cell walls. They suggested that more lipophylic flavonopidsexert antimicrobial activity by disruptiog microbial cells membranes.

The results of this study show that Phyllanthus amarus appreciable antimicrobial properties thus justifying its use as antimicrobial agent in Nigerian ethnomedicine. Parquetina thus justifying its use as antimicrobial agent in Nigerian ethnomedicine. Parquetina nigrens on the other hand was not as effective as P. amarus Howeve, caution must be exercised until pharmacists have adequately explored this extract in pharmaceutical preparation of antimicrobial agents. This is necessary in order to eliminate the non-useful aspect of the extract.

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