ANTI-TYPHOID PROPERTIES OF *PHYLLANTHUS AMARUS* EXTRACTS

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ABSTRACT

Phyllanthus amarus is a medicinal plant belonging to the family Euphorbiaceae and commonly known as 'carry-me-seed' or quinine weed. The whole plant was subjected to solvent extractions using petroleum ether and ethanol. Both crude extracts were tested for antimicrobial activity against Salmonella typhi using agar well-diffusion method of sensitivity testing. The crude ethanolic extract showed good inhibitory effect against the bacteria but the petroleum ether extract showed no activity. The crude ethanol extract was subjected to column chromatographic separation using dichloromethane: ethyl acetate (DCM/EA) solvent system. The column was finally eluted with methanol. The fractions eluted from the column were tested against the Salmonella typhi. The organism was sensitive to the methanol fractions at different concentrations (4.37mg/ml, 8.75mg/ml, 17.50mg/ml, 35.00mg/ml and 70.00mg/ml) with a zone of inhibition of 8mm, 12mm, 16mm, 20mm, and 22mm respectively. The Salmonella typhi was insensitive to the DCM/EA fractions. Phytochemical screening tests performed on the crude ethanolic extract revealed the presence of alkaloids, steroids, saponins, lignans, tannins and flavonoids.

INTRODUCTION

Typhoid fever is an enteric fever caused by *Salmonella typhi* (Parry, 1998; Parry *et. al*., 2002). The disease remains endemic in many developing countries and if not treated appropriately has a mortality of up to 30% (Pang *et al*., 1998). It results in over 20 million hospital cases annually, with at least 700,000 deaths (Edelman *et al*., 1986). The main burden of the disease is in developing countries, particularly in Africa. The emergence and widespread distribution of drug resistant *Salmonella typhi* have imposed serious limitations on successful antibiotic treatment (Ling, 1984). This has necessitated the search for alternative therapeutic agents effective against the organism from medicinal plants. The problem of antibiotic-resistant organisms results in increased hospitalizations, health costs, and mortality. It has therefore become an important public health hazard associated with serious consequences for the treatment of infections. To contain the problem of antimicrobial resistance, the World Health Organization has provided some interventions, one of which includes the continuous search for novel compounds from plant origin (Butler, 1973; Chen, 2004).

Phyllanthus amarus traditionally employed in the management of typhoid fever, has been found to be of high medicinal value and it is widely distributed in all tropical regions. The plant may be indigenous to the tropical Americans (Ayiku, 1992; Sittie *et al*., 1998). It is an erect annual herb and has a long history of use in folklore for the treatment of liver, kidney and bladder problems, diabetes, intestinal parasites, appendicitis and prostate problems, and bacterial infections (Schwontkowski, 1993). In Ghana, the plant has enormous medicinal uses in spite of it being a weed. Akobundu *et al*. (1998) reported the use of the entire plant in treating malaria, typhoid fever, skin infections in children as well as inducing labour in pregnant women. The green stem is a remedy for cough, hiccup and also prevents vomiting when chewed (Sittie *et al*., 1998). It is also used as an appetizer and also to treat protozoal infections (Robineau, 1991).

This study was to extract and isolate anti-typhoid bioactive principles in the ethanolic extract of *Phyllanthus amarus* using a bioassay guided technique and to determine the number of compounds present in the bioactive fractions using TLC analysis.

MATERIALS AND METHODS

Plant collection and treatment

Plant collection was made at Ntranoa, a village near Ankaful Prisons in the Central Region of Ghana and authenticated with the Voucher Specimen Number CCG 5152 at the School of Biological Sciences herbarium, University of Cape Coast. Soil particles were removed from the roots by washing and the entire plant was dried for 5 days. The dried plant sample was milled into powder using a heavy-duty blender and weighed into a clean zip lock plastic bag and labeled.

Extraction

One hundred grams of the powdered plant sample was successively cold extracted using 200ml each of petroleum ether and ethanol. The bulked petroleum ether and ethanol extracts were concentrated under vacuum using the rotary evaporator and kept in a desiccator. Thin layer chromatography of the crude ethanol was performed to investigate the chemical profile of the extract. Column chromatographic separation of the ethanolic extract using the solvent system (Dichloromethane/Ethyl Acetate, DCM/EA) and TLC monitoring yielded four fractions. These were concentrated and dried completely in a desiccator. Methanol was used to wash down the remaining components from the column. The DCM/EA and methanol fractions were subjected to antimicrobial susceptibility test.

Phytochemical analysis

Phytochemical analysis was carried out using the crude ethanolic extract with the standard methods of Harbone (1984) and Stahl (1984). Secondary metabolites including saponins [Frothing test], sapogenins [Antimony chloride spray; Acetone: Hexane 1:4 as solvent], steroid and terpenoids [Leibermann Burchard], flavonoids [Saturated alcoholic sodium acetate spray; Chloroform-Acetic acid- Water (90:45:6) as solvent], alkaloid [Dragendroffs test], phenolics (Ferric chloride test) and amino acids (Ninhydrin test) were present.

Thin layer Chromatographic analysis of methanol fraction

The chemical profile of the compounds present in the methanolic extract was determined using thin layer chromatography. Dichloromethane and ethyl acetate solvent system was use with different solvent ratios (Figure 2).

Antimicrobial susceptibility testing

The *Salmonella typhi* cultures used for the study were clinical isolates obtained from the medical laboratories of Kole-bu Teaching Hospital and were identified by standard bacteriological methods as described by NCCLS (2000). Well diffusion method using Müeller-Hinton agar plates were used to demonstrate the antimicrobial properties of the *Phyllanthus amarus* crude extracts (Khan *et al.*, 1988). A 100µl of bacteria suspension was aseptically introduced and spread using cotton swabs on surface of gelled sterile Muller Hilton agar

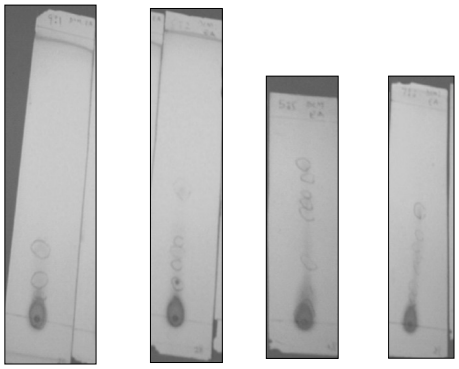

Plate 1 DCM: EA *Plate 2 DCM: EA* *Plate 3 DCM: EA* *Plate 4 DCM: EA*

Fig 2: TLC of methanol fraction using different solvent systems

plates. A well of about 6.0mm diameter with sterile cock borer was aseptically punched on each agar plate. A 50 µl of 4.375mg/ml, 8.75mg/ml, 17.535mg/ml, and 35.0 mg/ml and 70.00mg/ml concentrations of DCM/EA and methanol fractions were introduced into the wells aseptically. Plates were kept in laminar flow hood for 30 minutes for pre diffusion of extract to occur and later incubated at 37ºC for 24 hours. A negative control well too was made with 50µl of methanol. A positive control was made by placing antibiotic disc (Ciprofloxacin) on agar plate. Resulting zone of inhibition was measured and recorded. The culture plates were prepared in triplicates and the mean inhibition zone recorded.

RESULTS

Preliminary antimicrobial investigation of the crude petroleum ether and ethanolic extracts of *Phyllanthus amarus* on *Salmonella typhi* was done. The petroleum ether extract showed no activity on the bacteria but the ethanolic extract was active. Antibacterial activity of different concentrations of DCM/EA and methanolic fractions against the *Salmonella typhi* is presented in tables 1 and 2. The results showed that all the DCM/EA fractions were inactive on the bacterial strain (Table 1). The methanolic fraction of different concentrations showed activity to the *S. typhi*. Doubling of the concentration of the methanolic fractions showed a corresponding increase in the inhibition zone (Table 2). The result of preliminary phytochemical analysis of the ethanolic extract of the *P. amarus* indicated the presence of steroids, terpenoids, alkaloids, amino acids, phenolic and flavonoids and saponins, as major phytochemicals.

DISCUSSION

The World Health Organization (WHO) is encouraging, promoting and facilitating the effective use of herbal medicine in developing coun-

Table 1: Antibacterial activity of DCM/EA extracts of *Phyllanthus amarus* against S*almonella*

DCM/EA Fractions	Zone of inhibition at different concentrations (mg/ml)				
	5.00mg/ml	l9.50mg/ml	17.40mg/ml	40.60mg/ml	55.50mg/ml
1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	0	0	0
4	0	0	0	0	0
DCM/EA (negative control)	0	0	0	0	0

DCM/EA- dichloromethane/ethyl acetate

Table 2: Antibacterial activities of methanol fractions of *Phyllanthus amarus* against *Salmonella typhi*

Concentration (mg/ml)	ZONE OF INHIBITION(mm)
4.375	8
8.75	12
17.5	16
35.00	20
70.00	22
Methanol (negative control)	0
Ciprofloxacin disc (positive control)	17

tries for health programs. The potential of higher plants as a source of new drugs is still largely unexplored. Hence, the last decade has witnessed an increase in the investigations of plants as a source of new biomolecules for human disease management (Mohana *et al*., 2008).

In vitro evaluation of plants for antimicrobial property may be considered to be among some of the first step towards achieving the goal of developing eco-friendly management of infectious diseases of humans by searching new biomolecules of plant origin (Mohana *et al*., 2008). Considering these, *Phyllanthus amarus* was screened *in vitro* for antibacterial activity against *Salmonella typhi.* On the basis of zones of inhibition, the results of the present investigation revealed that the DCM/EA fraction showed no antimicrobial activity on the bacteria. On the other hand the methanol fraction showed very promising results. The methanol fraction was active on the bacteria at different concentrations. The zones of inhibition obtained for the various methanol fractions were 22, 20, 16, 12 and 8 mm. The fraction with the highest concentration (70.00 mg/ml) gave the highest zone of inhibition of 22 mm and the fraction with the lowest concentration (4.375 mg/ml) gave the lowest zone of inhibition of 8 mm. This means that the higher the concentration of the plant extract, the higher the zone of inhibition and the lower the concentration of the fraction, the lower the zone of inhibition. Methanol and DCM/EA solvent mixture served as negative control whilst ciprofloxacin which is currently the antibiotic of choice for the treatment of typhoid fever served as positive control. Comparing the zones of inhibition of the methanol fractions to that of ciprofloxacin, *Salmonella typhi* was susceptible to the highest concentration (70.00mg/ml) of methanol fractions.

Further investigation on methanol fraction revealed that the methanol fraction was a mixture of components. This was demonstrated by chemical profile on thin layer chromatography. Phytochemical analysis of the ethanolic extract revealed the presence of alkaloids, flavonoids, steroids, saponins and tannins.

CONCLUSION

Phyllanthus amarus is very a potent medicinal plant against bacterial action (Jelager *et al*., 1998). The plant has anti-typhoid properties and the active principles may be found in the methanol fraction which was isolated from crude ethanolic extract. This has confirmed and justifies the use of *Phyllanthus amarus* as a herbal preparation in the treatment of typhoid fever. The crude ethanolic (polar) extract was also found to contain various amounts of phytochemicals such as saponins, steroids, flavonoids, amino acids, phenolic compounds and alkaloids which are known for their therapeutic effects.

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